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SOME PARALLELS BETWEEN GENE CONTROL SYSTEMS IN MAIZE AND IN BACTERIA

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It has been realized for some time that, although the gene is necessary for expression of a certain phenotype, it may not in itself be sufficient for such expression and mechanisms may exist that control its action. Genetic systems that serve this purpose in maize were recognized some years ago, and studies conducted with a number of them have been reported (for references, see Brink, 1958, 1960; McClintock, 1956a and b; Peterson, 1960). Without adequate confirmation of similar systems in other organisms, it could be considered that the systems in maize may not reflect a type of control of gene action that is common to organisms in general. Recently, however, genetic systems that control gene action have been discovered in bacteria (Jacob and Monod, 1959, 1961; Jacob *et al.*, 1960) and it is now apparent that a relationship may exist between the bacterial and the maize control systems. The bacterial control systems, described by Jacob *et al.*, are composed of two genetic elements, each distinct from the "structural" gene. One of them, designated the "operator," is located adjacent to the structural gene (or sequence of structural genes) and controls its activation. The structural gene, when activated, is responsible for the production of a particular sequence of amino acids and thus for the specificity of a protein. The second element of this system, termed the "regulator," may be located close to the structural gene, or it may be located elsewhere in the bacterial chromosome. The regulator is responsible for the production of a repressor substance—not a protein—that appears in the cytoplasm. The operator element responds in some yet unknown manner to changes in degree of effective action of the repressor substance by "turning on" or "turning off" the action of the structural gene in accordance with such changes. Each operator-regulator system is specific, in that an operator will respond only to the specific product of the regulator of its system.

In maize likewise, some of the control systems are composed, basically, of two elements. One is closely associated with the structural gene and directly controls its action; it may be likened to the operator element in bacteria. The other element may be located near the first or may be independently located in the chromosome complement. It establishes the conditions

to which the gene-associated element responds, a particular change in these conditions being reflected in a particular change in action of the gene, and thus is comparable to the regulator element in bacteria. In maize, as in bacteria, each "operator-regulator" system is quite specific: an "operator" element will respond only to the particular "regulator" element of its own system.

Several different two-element control systems, each operating independently of the others, have been identified in maize. These were discovered, originally, because the elements belonging to each were transposed from one location to another in the chromosome complement without losing their individual identities in the process. It was found that the gene-associated element of a system can leave the locus of one gene and become associated with that of another. After such an association is established, the action of the gene comes under the control of the system to which the gene-associated element belongs. It has been possible, therefore, to examine the mode of operation of a particular control system at a number of different gene loci and, conversely, to examine the operation of different control systems at the same gene locus. It should be emphasized that, although transposition of controlling elements in maize made it possible to recognize their presence in the chromosome complement and to study the mode of operation of the component elements of a system, transposition does not necessarily characterize the behavior of a controlling element. An element previously exhibiting transposition may become fixed in location. If it is the gene-associated element that becomes fixed, the action of the gene will then be permanently under the control of the system to which that element belongs. Examples will be considered in this report.

Jacob (Jacob, 1960; Jacob, Schaeffer and Wollman, 1960) and Richter (1961) have postulated that controlling elements in maize may be comparable to episomes in bacteria. Recent evidence (Buttin, Jacob and Monod, 1960; Yarmolinsky and Wiesmeyer, 1960) about the manner in which a phage particle may control the action of bacterial genes in the neighborhood of its attachment to the bacterial chromosome lends support to this interpretation. In a lysogenic bacterium, induction of phage by ultraviolet light or by chemical treatment releases inhibition of gene action not only in phage genes that are concerned with initiating vegetative replication but also in genes of the bacterial chromosome in the neighborhood of phage attachment. This effect resembles that which occurs in maize when a controlling element at the locus of a gene is removed by the transposition mechanism. A change in action of the gene accompanies this removal.

Notwithstanding the analogies that may be drawn between controlling elements in maize and episomes in bacteria, it now appears to the author that control systems in maize also resemble the operator-regulator systems of control of gene action in bacteria, as outlined above. In maize, as in bacteria, the controlling element (the "operator") at the locus of the structural gene responds to altered activities of the second element (the "regulator") of the system by inducing modification in action of the structural gene. In

maize, the response of the "operator" element to change in effective action of the "regulator" element results in controlled types of change in action of the structural gene, and many such changes are accompanied by removal of the "operator" element from the locus. In other cases, however, the "operator" element is not removed from the locus. It responds merely by "turning on" or "turning off" the action of the structural gene. When this occurs, the maize systems resemble the operator-regulator systems in bacteria.

The control system composed of the elements Dissociation (*Ds*) and Activator (*Ac*) was the first of those in maize to be explored extensively. Its mode of operation was examined at a number of different gene loci (McClintock, 1953). This system was studied intensively because it was possible to identify readily both the "operator" element, *Ds*, and the "regulator" element, *Ac*. In several cases (bz^{m-4}, sh^{m}, c^{m-1}) the proximal or distal position of *Ds* with respect to the components of the structural gene could also be determined. With some other two element systems, the regulator element is readily identifiable but the presence of the operator element at the gene locus often must be assumed on the basis of the control that the regulator exerts on gene action. However, when adequate test methods are available, it is possible to confirm the presence of an operator element at the locus of the gene by means of crossover techniques which are capable of defining its location with respect to the components of the structural gene. This method has been used successfully to define the location of this element in the case where action of the structural gene, A_1, came under the control of the two-element system of which Dotted (*Dt*) is the regulator (Laughnan, 1955; Sarma, 1956, 1961).

This report will describe the mode of operation of the elements that compose a single control system in maize. It is not the purpose of the paper to present the evidence for the statements that will be made here but rather to indicate some of the resemblances between the systems in bacteria and those in maize. The Suppressor-mutator control system in maize has been chosen because it illustrates these resemblances more directly than do other examined systems in maize.

Five independent inceptions of control of gene action by the Suppressor-mutator (*Spm*) control system have been recognized in the Cold Spring Harbor cultures. Three of them occurred when the "operator" element of this system was inserted at the locus of A_1 in chromosome 3. These three cases are designated a_1^{m-1}, a_1^{m-2}, and a_1^{m-5}. The symbols m-1, m-2, and m-5 refer to the order in time of inception of control of gene action at A_1 by this system (a_1^{m-3} and a_1^{m-4} refer to inceptions of control of gene action at A_1 by the *Ds-Ac* control system). A fourth case occurred at the A_2 locus in chromosome 5 (designated a_2^{m-1}), and a fifth at the *Wx* locus in chromosome 9 (designated wx^{m-8}). (Both A_1 and A_2 are associated with anthocyanin pigment formation in plant and kernel. *Wx* is associated with production amylose in the pollen grain and in the endosperm of the kernel.) Two independently located elements are primarily responsible for control of gene action at a_1^{m-1}, a_1^{m-5}, a_2^{m-1}, and wx^{m-8}. One controlling element, com-

parable to the operator, resides at the locus of the gene and directly controls its type of action. The other element, comparable to the regulator, is *Spm*, to which the "operator" element at the locus of the gene responds in accordance with the type of activity of *Spm* and the changes in this activity. Preliminary evidence suggests that *Spm* resides close to the A_1 locus in the case of $a_1{}^{m-2}$. It is possible that here the "operator" and "regulator" elements of the system are located adjacent or close to each other. If this proves to be true, $a_1{}^{m-2}$ will resemble in its organization and its behavior one of the cases examined in studies of the *Ds-Ac* system. (See discussion of bz^{m-2} in McClintock, 1956 c.)

Basically, the mode of control of gene action by the *Spm* system is relatively easy to comprehend. However, there are some conditions that complicate its analysis and so obscure its basic simplicity. They arise from (1) alterations at the locus of the gene (termed "altered states" of the gene locus), induced by the controlling element there residing, that modify subsequent expression of the gene, both in the presence and in the absence of *Spm*; (2) modifications of *Spm* itself, expressed by altered degrees in strength of its action, or by cyclically occurring change in phase of its activity—from active to inactive and back to active; and (3) the action of an independently located, transposible Modifier element that alters the expression of some of the states of the gene locus in a predictable manner, but only when *Spm* is also present and in its active phase. Each of these conditions will be considered in turn. The discussion will apply to those cases in which the two elements that are basically concerned in control of gene action are independently located, one being at the locus of the gene, the other, *Spm*, being located elsewhere.

THE CLASS I AND CLASS II STATES OF A GENE LOCUS UNDER THE CONTROL OF THE *Spm* SYSTEM

There are two main categories of state, designated class I and class II. Because the class II states behave in a simple manner, they will be considered first. In the presence of a fully active *Spm*, no gene action is expressed. If *Spm* is removed by somatic transposition, or by meiotic segregation, or if it enters its inactive phase in a cell of the plant or the kernel, gene action is expressed. The degree of expression serves to distinguish between different members of the class II states. An apparently full, or near full gene expression characterizes some class II states whereas a much reduced expression characterizes others. With the class II states, *Spm* serves as the "regulator" of action of the "structural" gene, causing it to be "turned on" and "turned off" through the direct mediation of the "operator" element residing at the locus of the "structural" gene. It should be emphasized that this turning on and turning off of gene action is not accompanied by any modification that permanently alters the structure of the gene locus, as may occur with the class I states. However, the class II states originate from the class I states, which will now be described.

Because of the variety of expressions that may be produced by class I states, they appear to be far more complex than the class II states. Regardless of the degrees of difference in expression, all class I states exhibit the same basic pattern of behavior. With respect to any one gene under the control of the *Spm* system, all the many different class I states that have been isolated trace their origin to the class I state that was produced, initially, when the "operator" element became associated with the structural gene. Each class I state is distinguished not only by its behavior pattern in the presence of active *Spm*, but also by the type of gene action that it gives rise to in the absence of *Spm* (or in the presence of *Spm* in an inactive phase). When a fully active *Spm* is present, all gene action is suppressed until, in a cell of the plant or of the kernel, a modification is instigated at the locus of the gene by the "operator" element there residing. Each class I state is distinguished by a particular type of consequence of such modifications. There are two main consequences: production of a mutant, which is thereafter stable in the presence of active *Spm*; or production of a new state, either class I or class II. The time during development of a plant or kernel when these modifications occur, the number of cells in which they occur at any one stage in development, and the particular types of consequence, serve to characterize a particular class I state. For example, with some states, such modifications at the locus of the gene may occur early in development, but each state is distinguished from others by the type of consequence of these modifications. One such state may give rise to two main types of stable mutants, those that express high levels of gene action and those that give the null expression; and these two types of mutants are produced at constant relative rates. With another such state, the early-occurring mutations result only in mutants that express low levels of gene action. Some class I states give rise to many new states whereas others produce few new states.

There is a group of class I states characterized by the fact that all mutation-inducing events occur late in development of plant or kernel. The states in this group may be distinguished from one another by differences in the number of phenotypically distinguishable mutant areas that are produced, and also by the levels of gene action these mutant areas exhibit. These states are particularly useful for many studies. Mutation-inducing events occur so late in development that germinal mutations may not be encountered or are encountered only rarely. Thus, such states are preserved, unaltered, through generations of plants, even when *Spm* is present and fully active in the plants.

In the absence of *Spm* all but one of the many class I states that have been isolated express some degree of gene action. The level of action may be low with some states, intermediate with others, and even quite high with still others. As long as *Spm* is absent (or is present in its inactive phase), the particular type of gene action expressed by any one state is constant and may be maintained unaltered from generation to generation. Also, in the

absence of an active *Spm*, any state behaves as a stable allele of any other state. However, no relation has been observed between the type of gene expression that a class I state exhibits in the absence of *Spm* and the types of mutation it produces in its presence.

If, in a plant having an active *Spm*, a different class I state is carried in each homologue, each state reacts to *Spm* in its own individual manner, and each may be recovered in the progeny, unaltered by its association in the same nucleus with the other state. Again, if two different gene loci, each under the control of the *Spm* system, are present in a plant or a kernel—for example, a_2^{m-1} and wx^{m-8}—each responds to *Spm* in its own characteristic manner according to its state. It may be added, also, that in plants or kernels having two such states each state responds directly to a somatically occurring change in action of *Spm*. Each depicts this change in the expected manner, in accordance with its state (see below).

TYPES OF CHANGE IN *Spm*

Spm itself undergoes modification. After a modification has occurred, the *Spm* exhibiting it may be isolated and further examined. The modifications may result in one of several different types of change in *Spm* action or behavior. One type affects the time during development of the plant when transposition of *Spm* will occur. Some isolates of *Spm* undergo transposition mainly early in development. Others undergo transposition mainly late in development, with only an occasional occurrence in young tissues. Several isolates have been obtained that rarely undergo transposition at times during development that will result in the appearance of gametes in which *Spm* occupies a new location in the chromosome complement; and one isolate has not yet given any evidence of transposition.

One conspicuous type of change undergone by *Spm* results in a weakening of its capacity to effect mutation with the class I states of those gene loci that have come under the control of the *Spm* system. The designation Spm^w is used to symbolize this type of alteration of *Spm* (McClintock, 1957). In this section, the symbol Spm^s will henceforth be used to designate an *Spm* expressing full activity. A newly arising Spm^w may be recognized readily. With the class I states, its presence is made evident by a pronounced delay in time of occurrence of mutation at the locus of the gene, and also in a pronounced reduction in frequency of occurrence of such mutation. If the class I state is one that gives only late-occurring mutations with Spm^s, then with Spm^w only a few very late-occurring mutations will be produced, and sometimes none at all. If the class I state is one that gives many early-occurring mutations with Spm^s, then Spm^w will delay the time of occurrence of mutation until the late stages of development of a tissue. Only small areas exhibiting the mutant phenotype will appear in plant and kernel. The response of any one class I state to any one isolate of Spm^w is quite predictable.

Each Spm^w arises from an Spm^s as the consequence of a single event occurring within a cell. If it takes place early in plant development, all the

cells producing an ear of the plant may be descendants of the cell in which it occurred, and thus all carry the newly produced *Spm*^{w}. Or, the descendent cells may contribute only to a part of the ear, and the newly produced *Spm*^{w} be evident only in the kernels within a sector derived from these cells. In either case, the *Spm*^{w} may be isolated from those kernels that carry it. There are different types of *Spm*^{w}, distinguishable from each other by several criteria. They differ in degree of weakening of the capacity to induce mutation at the loci of genes controlled by the *Spm* system, in frequency of occurrence of transposition and the time of its occurrence during development, and in stability of the *Spm*^{w} expression. In the last-named respect, differences between *Spm*^{w} isolates of independent origin are conspicuous. Some isolates are highly stable whereas others undergo frequent return to *Spm*^{s}. It is again evident that change in action of *Spm*, this time from *Spm*^{w} to *Spm*^{s}, is effected by a single event occurring in an individual cell.

If both an *Spm*^{w} and an *Spm*^{s} are present in the same plant, *Spm*^{s} is dominant. However, the *Spm*^{w} in such plants may be recovered in their progeny, with its type of action unaltered by previous association in the same nucleus with *Spm*^{s}.

It should be emphasized that no modifications resulting in mutation or change in state will occur at the locus of a gene that is under the control of the *Spm* system (that is, with the class I states of the gene locus), unless all gene action has first been suppressed by *Spm* in the ancestor cells, whether by *Spm*^{w} or by *Spm*^{s}. In other words, suppression of gene action by *Spm* must precede the mutation-inducing event. This fact is especially well illustrated in plants that have an *Spm* element that is undergoing change in phase of its activity during the development of plant or kernel, as described in the next section.

CYCLICALLY OCCURRING REVERSALS OF PHASE OF ACTIVITY OF *Spm*

One of the most interesting and theoretically important types of expression of *Spm* consists in the sequentially occurring reversals in phase of its activity—from active to inactive and back to active (McClintock, 1958, 1959). Each such change in phase results from an event occurring in an individual cell of the plant or kernel. The effect produced by the change is then exhibited in the descendants of this cell, if either a class I or a class II state of a gene locus under the control of this *Spm* system is also present to register it. Following such a reversal of phase, the duration of the particular phase may be long, continuing unaltered through many cell or even plant generations, or it may be short, reversal occurring again in a number of cells only a relatively few cell generations removed from that which initiated the preceding phase. Control of duration of a particular phase appears to be associated with the event that produces the particular reversal of phase. By selective methods it has been possible to isolate *Spm* displaying either a long duration of an active phase or a long duration of an inactive phase.

The phenotypes appearing in mature plants and kernels as the consequence of phase reversal of *Spm* may be very complex. The degree of complexity depends on the state of a gene locus that is present in the plant or kernel, on the number of reversals of phase of *Spm* that occur, and also on the times of their occurrence during development of a tissue. If an inactive *Spm* is present, initially, in a plant or kernel, along with either a class I or a class II state of a gene locus, no evidence of the presence of this *Spm* will appear in either the plant or the kernel unless reversal of phase occurs in one or more cells during development. After *Spm* is reactivated in an individual cell, its presence is revealed in the descendants of that cell. When a class II state is used as the indicator, reactivation of *Spm* is made evident by suppression of gene action in these cells. Should subsequent reversal of phase occur in some of the descendent cells, then gene action will be evidenced in their descendent cells. With the class II states, then, reversals of phase of activity of *Spm* merely effect a "turning on" and "turning off" of gene action; and with the class II states of those gene loci that are associated with the production of anthocyanin pigment, both in plant and in kernel, the alternating cycles of phase of activity of *Spm* are registered with great clarity. No pigment appears in cells in which *Spm* is in its active phase, and pigment appears in those cells in which it is in its inactive phase. It may be pointed out here that the type of control of gene action, just described, resembles that associated with phase variation in Salmonella in which the system of chromosomal elements responsible for control of gene action likewise has been identified (Lederberg and Iino, 1956; Iino and Lederberg, 1957, 1958; Iino, 1959, 1960).

The response of the class I states to reversal of phase of activity of *Spm* is basically the same as that of the class II states, but the types of phenotypic expression of the gene and the various different patterns of expression that may appear in an individual plant or kernel can be very complex. This is because mutation-inducing events may occur at the gene locus in some cells when their *Spm* is in its active phase. The pattern of mutant areas that may appear in a sector of the plant or kernel, after a change in phase of *Spm* from inactive to active, will depend upon the developmental stage of the tissue when the reversal occurs. An example will illustrate this. If a plant or kernel starts development with an active *Spm* having a long duration of the active phase, and also a class I state of the gene locus that responds to it by producing a number of early-occurring mutations, then large areas, each exhibiting a mutant phenotype, will be present in the mature plant or kernel. Each such area reflects an early-occurring "operator"-induced modification at the locus of the gene. If, however, development commences with *Spm* in its inactive phase, no mutations may occur at the gene locus having this class I state unless and until a reversal of phase of activity of *Spm* occurs. If reversal takes place in a cell rather late in the development of a tissue, suppression of gene action will be effected in the descendants of that cell. However, mutation-inducing events may occur in some of these descendent cells and, by necessity, all these mutations will arise in cells

of a tissue that is approaching maturity. Consequently, the areas that can exhibit a mutant phenotype must be small. In other words, the size of the mutant areas will depend upon the stage of development of a tissue when *Spm* reverts to its active phase. It is evident, then, that complex and often irregular patterns of gene expression may be exhibited by a plant or by a kernel carrying a particular class I state when its *Spm* is undergoing frequent reversal of phase of activity. Different patterns of gene expression may be observed in different areas of the same plant or kernel. These patterns reflect the time of occurrence of reversal of phase of activity of *Spm* and also the number of such reversals.

Evidence has been obtained to indicate that inactivation of *Spm*, as described above, is not associated with a complete blocking of its functional capacity but rather with some change affecting its mode of functioning, such as an altered form of its product. This was made evident, initially, in plants and kernels having an inactive *Spm* characterized by a long duration of inactivity, and also an active *Spm* undergoing frequent reversal of phase during development. If only the latter *Spm* were present, a class II state would register each reversal of phase by showing no evidence of gene action in those cells in which it was active and by exhibiting gene action in those cells in which it was inactive. When two active *Spm* elements are present, initially, a class II state registers reversal of phase only when it occurs to both *Spm* elements, either simultaneously in an individual cell, or successively (that is, affecting one *Spm* in one cell and the other *Spm* in a descendant of that cell). Thus, in either plant or kernel, both the number and the size of areas exhibiting gene action will differ according to the number of active *Spm* elements that were present initially. From these patterns, it is often possible to deduce the number of *Spm* elements that are present in a plant or kernel.

It was anticipated that combination of an inactive *Spm*, having a long duration of the inactive phase, with an initially active *Spm* in a plant or kernel carrying a class II state would give rise to a phenotype resembling that produced when only one active *Spm* is present initially. This assumption proved to be incorrect. Instead, it was found that this combination produced a phenotype resembling the one that appears when two active *Spm* elements are initially present in a plant or when three active *Spm* elements are initially present in a kernel. However, the pattern produced by the areas that exhibit gene action (no active *Spm* in them) is much more uniform, and this is particularly well illustrated in the aleurone layer of kernels whose endosperms receive two inactive *Spm* elements from the female parent and one initially active *Spm* from the male parent. All areas exhibiting gene action are small, and they are evenly distributed over the aleurone layer. That this pattern is not produced by reversal of phase of the inactive *Spm*, brought about by association in the same nucleus with an active *Spm*, is made evident when progeny of plants having an initially active *Spm* and the described inactive *Spm* are examined. The inactive *Spm* is recovered with its phase quite unaltered. Also, it appears in the expected proportions of the progeny

in accordance with the type of testcross that has been made to determine this. In order to be certain that the inactive *Spm* appearing in the progeny was the same as that which had been combined with the active *Spm* in the zygote produced from the initial cross, the relative locations of the two *Spm* elements in the chromosome complement had to be known in advance of the initial cross. Also, the location of each had to be determined in the individual progeny.

A number of tests had been made to observe the effects produced on either the class I or the class II states by bringing together in a zygote nucleus, or in a primary endosperm nucleus, an inactive and an active *Spm*. The effects produced in all such tests conformed with that described above. The inactive *Spm* proved not to be totally inactive, although it was quite ineffective by itself. This evidence does not preclude the possibility or the probability that some modifications of *Spm* may result in its total inactivation.

THE MODIFIER ELEMENT IN THE *Spm* SYSTEM

A transposible element that serves to increase the frequency of occurrence of mutation-inducing events with some of the class I states of $a_1{}^{m-1}$ first appeared in only one of many $a_1{}^{m-1}$, *Spm*-carrying kernels on an ear and on only one of several ears produced by an $a_1{}^{m-1}/a_1$, *Spm*-carrying plant. This kernel exhibited a marked increase in mutation frequency in comparison with that exhibited by the other $a_1{}^{m-1}$, *Spm*-carrying kernels on the ear. The class I state of $a_1{}^{m-1}$ that was present in the ear-bearing plant was one that undergoes only late-occurring mutations in the presence of an active *Spm*. No change in this state had been observed to occur in many tests conducted with it over a number of plant generations. The presence of a Modifier element, which was responsible for the marked increase in mutation frequency in the exceptional kernel, was revealed in tests conducted with the plant derived from this kernel. Subsequently, the effects produced by this Modifier on the expression of other class I states of $a_1{}^{m-1}$ were investigated. Study of its effects was confined to $a_1{}^{m-1}$, but the results allow the following conclusions to be drawn:

(1) The presence of the Modifier can be detected only when *Spm* also is present in the chromosome complement and only when it is in its active phase. Under these circumstances, the Modifier effects a marked increase in frequency of mutation to stable alleles with some of the class I states, but does not modify the time of occurrence of such mutation. Also, the rate of increase in frequency of mutation is proportional to that produced by the state in the absence of the Modifier. However, if the state is one that produces very many mutations with *Spm* alone, the Modifier does not effect a measurable increase in mutation rate (McClintock, 1958).

(2) When the Modifier is present, the same phenotype is produced with Spm^w as with Spm^s. Thus, plants and kernels that have only Spm^w and the Modifier are not distinguishable in phenotype from those that have Spm^s and the Modifier. However, the presence of either one or the other type of *Spm* may be determined by means of progeny tests. Individuals carrying *Spm* but

no Modifier appear in the progeny, and the type of *Spm* in them is made evident.

(3) The Modifier element is transposible. A number of early-occurring transpositions of it were detected. Its transposition to and away from locations in the chromosome complement close to marked gene loci were examined (McClintock, 1958).

The Modifier element acts as if it could complement both the "regulator" element, *Spm*, and the element of this system that is at the locus of the gene. It complements *Spm* in that in its presence a weakly acting *Spm* (Spm^w) is as effective as a fully active *Spm* (Spm^s). It complements the element at the A_1 locus in that in its presence a class I state that gives relatively few mutations with Spm^s alone can mimic another state that gives many more mutations with Spm^s alone.

DISCUSSION AND SUMMARY

Although the mode of operation of the *Spm* system of control of gene action, as outlined above, may appear to be complex, it is evident, nevertheless, that the diverse gene expressions that it may produce stem from one basic mechanism of action and response of the component elements of the system. The action of the *Spm* element resembles that of the regulator "gene" in bacteria. It may well be that *Spm* produces a specific repressor substance to which the element of the system at the locus of the gene, the "operator" element, responds by "turning off" gene action. Suppression of gene action requires the presence of this operator element at the locus of the gene. No suppression occurs when an operator element belonging to another system is present at the gene locus, or when the specific operator element of this system is transposed away from the gene locus. In other words, if *Spm* produces a specific repressor substance, then the operator element at the locus of the gene responds only to this specific repressor and to no other. The same principle would apply to all the two-element control systems investigated so far in maize; and in this respect they resemble the two-element control systems in bacteria.

In bacteria, both the operator and the regulator element undergo mutation. The mutations arise from single events, and some of them are reversible. The same applies to the controlling elements in maize. Each can undergo mutation, and each such mutation is produced by a single event. Also, some of them are reversible.

In bacteria, most of the control systems that are subject to analysis effect control of production of specific enzymes in response to certain changes in the cellular environment. A "turning on" and "turning off" of gene action constitute an efficient means of control of production of enzymes in response to changes in intracellular environment. Mutations, such as those that occur with the class I states described above, would effect a differentiation. Some of them could be lethal or could result in competitive disadvantage for unicellular organisms. In higher organisms, such mutations, occurring in somatic tissues, need not be lethal or disadvantageous

and, indeed, may be required. Specific types of mutation occurring at given times during development and produced by a control system, such as the *Spm* system, may effect tissue differentiation along certain paths. However, as emphasized above, the *Spm* system can operate in either way—in a manner similar to that exhibited in bacteria, or in a manner that accomplishes a permanent and specific type of change in gene action.

The class II states of gene loci under the control of the *Spm* system best illustrate the similarities in mode of operation of the bacterial and the maize systems. The "operator" element is fixed in location. No transpositions of it away from the gene locus occur, nor does it effect mutation at the gene locus. Its behavior is much the same as that of the operator "gene" in bacteria. If *Spm* produces a specific repressor substance, then the operator element responds to this by "turning off" gene action. If the repressor substance is not produced, or if its structure is modified by mutations that occur to *Spm*, then gene action is "turned on." A class II state, with its operator element fixed in position and an *Spm* element that also is fixed in position, gives rise to a system of control of gene action in maize that simulates in its mode of operation some of the described systems in bacteria. As stated earlier, cases of effective fixation of *Spm* at a specific locus have been found.

Study of the *Spm* system has shown that a relatively simple system of control of gene action may be derived from one that originally expressed a seemingly complex pattern of such control and it may be no coincidence that this simple system resembles those recently discovered in bacteria and in phage. It is expected that such a basic mechanism of control of gene action will be operative in all organisms. In higher organisms, lack of means of identifying the components of a control system of this type may be responsible for delay in recognition of their general prevalence, even though there is much genetic and cytological evidence to indicate that control systems do exist. It is anticipated, however, that control systems exhibiting more complex levels of integration will be found in the higher organisms.

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A REMOTE COINCIDENCE*

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Basic experiments in cytogenetics depend on the principle of chromosome mapping. This alone makes possible the interpretation of heritable results in terms of cytological events. The principle of chromosome mapping rests in turn on other concepts:

(1) A chiasma is the "outward and visible" sign of a genetic recombination between two of a set of four chromatin strands.
(2) Chiasmata are formed at random along the length of the chromosome.
(3) The chance of a chiasma forming in a given region is proportional to the physical length of that region.

Perhaps these three concepts are better regarded as hallowed by tradition than established as axioms. They are constantly under review as experimental knowledge probes deeper. With regard to Concept (1), it is now difficult to harmonize the time of DNA replication, the time of genetic recombination, and the time at which chiasmata become visible (Taylor, 1957). Whether or not these three events are synchronous, it seems evident that there must be a 1:1:1:relationship between them. This follows from the two classic proofs of crossing-over supplied by Stern (1931) and Creighton and McClintock (1931). Concept (2), if applied *sensu strictu*, is patently false. Random chiasmata should produce a far closer correspondence between the genetic and cytological maps of the Drosophila chromosomes than is shown by the textbook illustrations. One can suppose, if one wishes, that chiasmata are "almost" random—or only random in the places where they happen to fit the cytological map. This is not a very productive approach. Alternatively, one can consider the possibility that chiasmata are produced according to some pattern and that this pattern may provide a satisfactory substitute for the random chiasma concept. Concept (3) follows as a natural sequel to Concept (2). It is usually accepted—with reservations. If it were strictly true, the chance of a simultaneous occurrence of two chiasmata should be the product of their individual probabilities. It is not true when the map distance between the chiasmata is short, because, then, the number of double crossovers actually produced is less than expected (*interference*). The amount of interference is usually measured as *coincidence*, that is, the ratio of the number of doubles obtained over the number of doubles expected. Interference in the past has been interpreted on a mechanical basis, a double break being difficult to achieve with a

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semirigid "rope" of a restricted length. But it has apparently been forgotten that interference may not be restricted to contiguous regions. Weinstein (1918)[1] made the interesting observation that interference, as measured by coincidence, decreased with distance only to increase again in regions more remote. This observation provided the title for this article and an idea for reinvestigating Concepts (2) and (3).

DIFFICULTIES OF THE RANDOM CHIASMATA CONCEPT

The first comment one can make on this concept is that it is unnecessary. Consider a situation in which one, and only one, chiasma occurs in a chromosome, and the position in which it occurs follows a normal distribution with a standard deviation sufficient to "scan" the chromosome from one end to the other. The map one would obtain from this hypothetical situation would place the genes in the same order as that obtained under the conditions of a single random chiasma and the map length would be identical. If the genes in this chromosome model were located at equal physical intervals, they would preserve those intervals in the random chiasma map. With a normally distributed chiasma, the genes would appear crowded toward the ends of the chromosome and more widely spaced in the middle. This is the situation one actually finds in the genetic maps of Drosophila chromosomes.

The occurrence of double and (occasionally) triple crossovers shows, however, that a single chiasma hypothesis is too simple and that at least three chiasmata must sometimes be formed. It does not follow that these extra chiasmata should be positioned at random; they, too, may be normally distributed with characteristic standard deviations, according to the model developed by Mather (1936).

How do these suggestions relate to interference? If the number of chiasmata formed per chromosome is not limiting, then interference must be accounted for solely by the relative distances between chiasmata. This will be true either for a "randomly distributed" or a "normally distributed" situation. However, if the number of chiasmata is strictly limited, interference will be exhibited which is not *directly* related to physical distance. Thus, if only a single chiasma is formed in a chromosomal arm, there will be absolute interference against double crossovers, quite independent of the physical lengths and distance apart of the intervals involved. Different degrees of interference will be expected if a second chiasma is formed occasionally, and the amount of interference will be inversely proportional to the frequency with which the second chiasma is formed. *It follows, then, that interference calculations are of little significance unless they are related to the total number of chiasmata per chromosome, or chromosome arm.*

There is considerable information, mainly obtained from general observation but partly from internal evidence, that the number of chiasmata per bivalent is rather strictly limited and probably under genetic control. One is struck by the fact that well-mapped chromosomes in classic genetic material rarely exceed 150 crossover units—equivalent to 1.5 recombinations per strand or 3.0 chiasmata per bivalent. Only the four longest chromosomes

[1]See also Gowen (1919).

in corn exceed 100 map units; the others vary from 28 to 96 (Rhoades, 1954). In other species which have been extensively mapped, the approximate ranges in map units per chromosome are:

Drosophila melanogaster	0–107	(Sinnott *et al.*, 1959)
Hordeum distichum	22–60	(Smith, 1951)
Pisum sativum	57–136	(Lamprecht, 1958)
Lycopersicon esculentum	11–98	(Rick and Butler, 1956)

The outstanding exception to the common range is the sex chromosome of *Habrobracon juglandis* with a map length of over 400 units (Martin, 1947).

The range of one to three chiasmata per bivalent determined from genetic map lengths agrees well with the range obtained by direct chiasma counts at diplotene:

	Mean chiasmata per bivalent	
Lilium spp.	3–5	(Mather, 1935)
Campanula	2.67	(Darlington, 1937)
Rosa	2.66	(Darlington, 1937)
Tulipa	2.13	(Darlington, 1937)
Stenobothrus	2.05	(Darlington, 1937)
Spirogyra	2.00	(Richardson, 1934)
Zea mays	1.70	(Beadle, 1933)
Melanoplus	1–1.77	(Hearne and Huskins, 1935)

Mean counts of this order imply that multiple recombinations above triples in most species may be so rare as to be of little biological significance. Direct proof of this would require the rearing of very large numbers of offspring from hybrids heterozygous in five or more linked loci well spaced on a long chromosome. Such studies are no longer popular. Perhaps the most extensive data which are readily available are those of Weinstein (1918) which were collected for another purpose (to be discussed later). Weinstein's data included a count of over 37,000 male fruit flies whose mothers were heterozygous for four or more linked markers on their X-chromosomes. Only four per cent double, and less than 0.1 per cent triple recombinations were recovered. Only 2,500 of these flies were marked in five loci and these yielded 0.2 per cent triples and no quadruples. In this material, therefore, the genetic consequences of triple and higher recombinations are only of academic interest.

Stebbins (1950) and Grant (1958) have pointed out the importance of recombination frequency in relation to breeding system and life form. Their arguments suggest strongly that chromosome number and chiasma frequency per bivalent have been built into an organized system in each species as a result of natural selection. A corollary feature of this concept would be that the restriction of chiasma frequency within a very limited and similar range represents an optimum situation for a wide variety of organisms. If in an individual organism the chromosome number is constant, the mean chiasma frequency fixed within narrow limits, and the breeding system established, it is a little difficult to imagine that the recombination of genes at each meiotic cycle is left uncontrolled.

Finally, there is the evidence that a restriction of recombination in one chromosome may be compensated by increased recombination in the remainder of the genome (Mather and Lamm, 1935; Schultz and Redfield, 1951). More recently there are preliminary data in corn and cotton (Rhoades and Dempsey, 1953; Stephens, in press) which can be interpreted as compensation in the proximal region of a chromosome for restricted recombination in a more distal region. These relationships, and more especially their specific details, are difficult to reconcile with the classic concept of random chiasmata formation.

THE DETECTION OF PATTERNS

1. *Location of chiasmata in the sex chromosome of Drosophila melanogaster*

The source material for this investigation was the forty-year old data of Weinstein which have been cited earlier in this article. The data are admirably suitable for analysis because they deal with recombination in X-chromosomes marked almost from end to end. Precise recombinations over the chromosome as a whole were recorded, and the counts were made on very large numbers. Weinstein used the data to compare coincidence values between a specific segment ("eosin-ruby") at the distal end of the chromosome and a series of suitably marked segments at progressively greater distances extending to "fused-cleft" near the centric region. He obtained evidence of interference not only with segments located near to "eosin-ruby" but also with the remote "forked-cleft" region. There was an optimum distance between crossovers (chiasmata): within and beyond this distance interference increased. Weinstein interpreted this pattern on the basis of optimal loop length between chiasmata. Triple crossovers were excluded from his calculations. Gowen (1919) using a different form of analysis reached similar conclusions in his study of the third chromosome.

These results are compatible with a modified system of random chiasmata formation. An optimal loop length would imply that neighboring chiasmata could, as it were, "slide tandemwise" along the chromosome, preserving an optimal distance between them. The length of the "slide" would depend on the ratio between the optimal distance and the length of the chromosome arm, and within these limits crossing-over could occur with equal frequency at any place. On the other hand, it remains possible that the chiasmata might tend to occupy fixed locations and the length of the "slide" might be very restricted. In other words, there might be not only an optimal distance between chiasmata, but also an optimal distance between the proximal chiasma and the centromere. Thus in a sequence, *a b c d e f*, in which the optimal distance is three letters, under the first hypothesis there should be no interference between any of three relative positions, *a-d*, *b-e*, *c-f*. Under the second hypothesis minimal interference should occur in only one position, say, *b-e*.

It has been possible to re-examine Weinstein's data and to discriminate between these two hypotheses. The method of analysis used is different because the usual method of calculating coincidence is inaccurate. If the

crossover frequencies in two segments are p and q, respectively, the expected frequency of double crossovers is their product, pq. Both p and q are estimates, often with high standard errors, and the errors are compounded in their product. A simpler test for interference was suggested to me by Dr. R. C. Lewontin (Stephens, 1955). If there is no interference, then the ratio of non-crossover classes to singles should not differ significantly from the ratio of singles to doubles:

$$(1-p)(1-q):p(1-q)=q(1-p):pq$$

This forms the basis of a simple contingency test with one degree of freedom. The χ^2 value is proportional to the amount of interference. The test does not discriminate between "positive" and "negative" interference, but in practice the latter occurs rarely and can be noted when the analysis is performed. This method could, theoretically, be extended to examine interference in triple crossovers:

$$p(1-q)(1-r):pq(1-r)=pr(1-q):pqr$$

But there are three ways of performing this contingency test, and because different degrees of interference in double combinations are confounded in the calculations, they provide different χ^2 estimates. Instead, the following relationship was used:

Non-crossovers : Mean of singles = Mean of doubles : Triples

The χ^2 value obtained is proportional to the amount of interference in the recovery of triple crossovers.

A re-examination of Weinstein's data for the X-chromosome using these methods is presented in table 1. The code numbers, "W-2" etc., identify the numbers of the tables in his (1918) paper which provided the data. For convenience of presentation the intervals between markers are designated by small letters in the tables. Different combinations of markers were used in Weinstein's experiments, so that the data in different crosses are usually not strictly comparable. Thus, for example, in table 1, the lozenge and sable markers were never included in the same cross, so that the interval, *c*, is always confounded with the neighboring intervals, *b* or *d*.

2. *The modal positions of double and triple crossovers*

If chiasmata are positioned at definite locations along the chromosome, there should be a specific pair of segments which exhibits minimal interference to double crossing-over, and a specific triad of segments in the case of triple crossovers. Alternatively, if the pattern is essentially random but modified by mechanical restrictions, there should be a close inverse relationship between distance between crossover points and amount of interference.

Considering double crossovers first, the data on the left of table 1 show that interference is significant in all combinations which involve a proximal and medial segment or a medial and distal segment. Combinations of proxi-

TABLE 1

Patterns of interference in the X-chromosome of *Drosophila melanogaster*. (Data from Weinstein, 1918.)

	Interval map of X c.o. distance	*a* eosin 1	*b* ruby 7	*c* lozenge 23	*d* sable 43	*e* forked 57	*f* fused 60	cleft 65	Centromere			
		Doubles							Triples			
W-2	Combinations tested	*a/bc*	*bc/d*	*a/d*					*a/bc/d*			
	χ^2 (interference)	96.69	115.45	0.18					42.05			
W-3	Combinations tested	*a/bcd*	*bcd/ef*	*a/ef*					*a/bcd/ef*			
	χ^2 (interference)	70.61	82.33	3.78					35.81			
W-5	Combinations tested	*a/b*	*a/cd*	*a/ef*	*b/cd*	*b/ef*	*cd/ef*		*a/b/cd*	*a/b/ef*	*a/cd/ef*	*b/cd/ef*
	χ^2 (interference)	18.65	3.98	5.75	52.50	1.48	12.05		5.05	0.07	0.60	9.41
W-7	Combinations tested	*a/bcd*	*bcd/e*	*a/e*					*a/bcd/e*			
	χ^2 (interference)	32.24	3.98	0.09					14.95			

Five per cent level of significance: $\chi^2 = 3.84$.

mal and distal segments usually, but not always, show no interference. The phenomenon is therefore not entirely an inverse function of distance.

The existence of a pattern in chiasma positioning is shown more clearly in table 2. Here the same data given in table 1 are arranged in order of map distance. It can be seen at once that the relation between distance and interference is only a very general one and that there is a minimal interference distance of about 50 crossover units. Greater distances tend to show

TABLE 2

A comparison of mean distance between double crossover points and amount of interference. (Data summarized from table 1.)

Segments involved	Mean distance apart	χ^2 (interference)
a/ef	57	3.78
a/ef	57	5.75*
a/e	54.5	0.09
a/d	46	0.18
b/ef	46	1.48
a/cd	36	3.98*
bcd/ef	29	82.33†
a/bcd	28	70.61†
a/bcd	28	32.24†
bcd/e	26.5	3.98*
b/cd	25	52.50†
bc/d	25	115.45†
a/bc	21	96.69†
cd/ef	21	12.05†
a/b	11	18.65†

*Significant at five per cent level.
†Significant at 0.1 per cent level.

some interference. On the other hand, some shorter distances show less interference than longer distances. When one considers specific segments, it becomes apparent that the three cases in which interference is absent entirely involve the segments, *a* or *b* and *e*. The data as a whole may be interpreted on the basis of a proximal chiasma with a modal position in the *e* region and a distal chiasma which tends to be located somewhere between *a* and *b*.

Turning now to the data on triple crossovers, on the right of table 1, three of the four families show strong interference (*P* less than 0.1 per cent). In effect this is merely a restatement of the fact that triple recombinations were rarely recovered in these families. In the fourth family, W-5, triple recombinations were twice as frequent (table 3) and the four marked intervals enable comparisons to be made. Minimal interference is found in the com-

bination, *a/b/ef*, which suggests that the modes of two of the chiasmata are located in approximately the same positions as in two-chiasmata bivalents, with a third chiasma added in a more distal position. The second and third (distal) chiasmata are positioned very closely together in terms of map units:

Xta position		*	*			*		
Markers	eosin	ruby	lozenge	sable	forked	fused	cleft	(C)
c.o. distance	1	7	23	43	57	60	65	

3. *The modal position of single crossovers*

Obviously minimal interference calculations are not applicable to single chiasma bivalents and more indirect methods have to be used. The method adopted here has been to remove the effects of two- and three-chiasmata bivalents from the genetic crossover data, to construct a "corrected" linkage map and to compare it with the map obtained from the "uncorrected" data.

TABLE 3

Relative frequencies of different crossover types in the X-chromosome of *Drosophila melanogaster*. (Data from Weinstein, 1918.)

Group	No. of marked intervals	No. of flies examined	*NCO*	Percentage		
				Singles	Doubles	Triples
W-2	3	12,387	51.36	43.80	4.79	0.05
W-3	3	14,175*	48.63	47.02	4.25	0.10
W-5	4	2,572	47.32	45.88	6.57	0.23
W-7	3	8,298	51.34	45.93	2.70	0.04

Heterogeneity: χ^2 (9) = 134; P < .001

*This figure is printed incorrectly in the original table.

In table 3, the same data which have so far been analyzed are presented as frequency distributions in which the relative proportions of non-crossover, single-, double-, and triple-crossover strands can be compared. For comparative purposes the data are shown as percentages with the total family size listed in each case. A contingency test (conducted on the raw data, not on the percentages) showed that the frequency distributions of the individual families were highly heterogeneous so that the data cannot be pooled for analysis. The heterogeneity may be partly ascribed to differences in multiple crossover frequencies, partly to a differential recovery of the non-crossover classes. (The effects of competition would be particularly strong in crosses involving multiply-marked X-chromosomes.)

Theoretically, the proportions of the different crossover classes should follow a binomial distribution, $(0.5 + 0.5)^n$, where n is the number of chiasmata per bivalent. If the relative frequencies of bivalents with one-chiasma, two-chiasmata, etc., are known, the expected frequencies of the genetic crossover classes can be determined (Mather, 1936). Conversely, the rela-

tive frequencies of the different orders of bivalents can be estimated from the genetic crossover classes. If these theoretical expectations hold, no combination of different orders of bivalent should produce an excess of *NCO* strands over singles. The upper limit is attained when only one-chiasma bivalents are formed leading to the production of *NCO*'s and singles in equal numbers. The inclusion of a number of two-chiasmata bivalents will reduce the *NCO* class appreciably. The data in table 3 tells us that the great majority of bivalents had one chiasma (near equality of *NCO* and singles classes, low frequencies of doubles and triples). The five per cent excess of *NCO* over single classes is due, presumably, to selection favoring the wild-type chromosome. This departure from expectation will be ignored in the analysis to follow, which is approximate only.

Consider a triad of chromosomal regions, *p*, *q*, and *r*, marked as in Weinstein's data, and involved in crossing-over in a typical four-stranded bivalent. The chance of more than three chiasmata forming in the bivalent is so rare as to be safely neglected. Three chiasmata are occasionally produced. They will produce the following eight types of strand in equal proportions:

NCO, p, q, r; pq, pr, qr; pqr

Of these only the triple, *pqr*, is genetically identified as resulting from a triple crossover. By subtracting the number of triples from the data and an equal number from the remaining classes, the effect of triple chiasmata bivalents will be removed.

The remaining data can be ascribed to the combined effects of double and single chiasmata bivalents. The double chiasmata will produce four types of strand in equal proportions in each of three sets:

(1) *NCO, p, q; pq*
(2) *NCO, p, r; pr*
(3) *NCO, q, r; qr*

Subtracting the number of doubles and an equal number from the remaining classes in each set, the effects of double chiasmata bivalents can be removed. The remaining data can be ascribed to the sole effect of single-chiasma bivalents.

The original data, before adjustment, can be used to construct a genetic map in the usual way. The data, after adjustment, will provide a relative map based entirely on single-chiasma bivalents. The "singles" map will be shorter than standard because of the reduced chiasma frequency, but *if chiasmata are produced at random*, the relative intervals between markers should not be altered.

In table 4 the foregoing method of analysis has been applied to Weinstein's data. In each of the four families, the upper map (a) is based on the total data, the middle map (b) is the relative "singles" map. The lower map (c) is the same as (b) but corrected to standard map length for comparative purposes. It can be seen that the "singles" map has expanded in the mid-region and contracted at either end as compared with the standard map.

TABLE 4

Relative maps of the X-chromosome of *Drosophila melanogaster*, (a) as determined from total data, (b) as determined after removing the effects of double and triple chiasmata, and (c) as in (b) but corrected for total map length. (Data from Weinstein, 1918.)

		Map intervals				Total
		a	*bc*	*d*		
W-2	(a)	5.08	33.28	13.55		51.91
	(b)	1.42	31.60	7.48		40.50
	(c)	1.82	40.50	9.59		51.91
		a	*bcd*	*ef*		
W-3	(a)	5.60	41.45	8.75		55.80
	(b)	2.49	40.31	3.75		46.55
	(c)	2.98	48.32	4.50		55.80
		a	*b*	*cd*	*ef*	
W-5	(a)	5.40	16.10	28.46	9.77	59.73
	(b)	3.18	12.20	24.82	4.54	44.74
	(c)	4.25	16.29	33.13	6.06	59.73
		a	*bcd*	*e*		
W-7	(a)	5.83	43.19	2.41		51.43
	(b)	2.40	43.59	0.58		46.57
	(c)	2.65	48.14	0.64		51.43

This means that a single chiasma has a modal position toward the middle of the chromosome. The modal position appears to be located at or near the *c* interval, that is, between lozenge and sable:

Xta position							
Markers	eosin	ruby	lozenge	sable	forked	fused	clefs	(C)
c.o. distance	1	7	23	43	57	60	65	

The data indicate that chiasmata in the X-chromosome of *Drosophila melanogaster* have definite modal positions and that there is a sharp change in pattern in switching from single to double chiasmata. There is little further change in pattern when triple chiasmata are involved. With single chiasmata, genetic recombination is more frequent in the mid-region; with double and triple chiasmata, recombination shifts toward the proximal and distal regions. The pattern of recombination is therefore a function of the relative frequencies of single and double chiasmata. It is believed that the replacement of a random chiasma concept by a partially localized concept may have rather far-reaching consequences on genetic theory. I shall attempt to indicate some of these possibilities in the following paragraphs.

PARTIALLY LOCALIZED CHIASMATA

1. *Interference*

If chiasmata are produced in modal positions, then the phenomenon of interference is based on the interaction of two independent variables: (1) the

relative positions of the three loci involved; (2) the relative frequency of occurrence of single and double chiasmata bivalents. If one of the marked loci occurs in the median region of the chromosome, crossovers in its neighborhood will always show interference with crossovers anywhere else in the chromosome. If one of the marked loci occurs either in the proximal or distal region of the chromosome, interference will be inversely proportional to the frequency of two-chiasmata bivalents which are formed.

2. *Noncorrespondence of genetic (linkage) and cytological (physical) maps*

If the great majority of bivalents form a single chiasma with a modal position in the median region of the chromosome, then crossing-over will be more frequent in the mid-region and less frequent in the proximal and distal regions. Genes which are separated by equal physical intervals will appear more widely spaced in the mid-region and more crowded elsewhere. This explains satisfactorily the noncorrespondence of genetic and cytological maps in the X-chromosome of Drosophila. If the pattern exhibited here is a general one, it may be predicted that all chromosomes which do not exceed 50 map units (that is, with a mean of one chiasma per bivalent) will exhibit similar discrepancies between their genetic and cytological maps. In longer chromosomes (100–150 map units) the effects of single and double chiasmata will tend to neutralize each other, though it is possible that their genetic maps may show alternating bands of "crowding" and "separation," the latter corresponding to modal positions of chiasmata. It is tempting, but perhaps unwarranted, to interpret the maps of the longer corn chromosomes on this basis.

3. *External compensation—the Schultz-Redfield effect*

It has been known for some time that the number of chiasmata *per nucleus* tends to remain constant. Increase in chiasmata frequency in one part of the genome tends to be compensated elsewhere (Mather and Lamm, 1935). In *Drosophila melanogaster*, blocking of crossing-over in X-chromosome made heterozygous for inversions increases crossing-over in the autosomes (Steinberg and Frazer, 1944) and vice versa (Schultz and Redfield, 1951). The pattern of compensation is not uniform—crossing-over is greatly increased in the centric and distal regions, with relatively little change in the mid-region. This pattern is exactly what would be expected if the compensatory increase is gained by an increase in double chiasmata bivalents at the expense of the single chiasma bivalents. Increased genetic crossing-over can only be obtained by an increase in chiasma frequency which will involve a sharp change in modal positions of chiasmata.

4. *Internal compensation*

In *Drosophila melanogaster*, inversions do not have to include the entire length of a chromosome arm in order to suppress completely crossing-over in that arm. I am grateful to my colleague, Dr. L. Mettler, for the suggestion that the meiotic chromosomes of Drosophila, which are minute in comparison with the salivary chromosomes, may be unable to realize the complex loop

pairings which occur in the latter. The presence of an inversion may therefore prohibit intimate pairing and the prophase bivalents may be held together by forces similar to those obtaining in somatic pairings. In such a situation internal compensation (that is, within the same bivalent) would be impossible.

In plants internal compensation *is* possible. In corn, Rhoades and Dempsey (1953) found that crossing-over within the loop of an inversion was usually genetically ineffective, owing to dicentric formation. But in the immediate proximal segment the loss in recombination was almost exactly compensated. A similar situation was found by Morgan (1950) and Russell and Burnham (1950) working with different inversions. In cotton (Stephens, in press) a distal nonhomologous region was compensated not in the immediate neighborhood as in corn but over a long region which apparently includes the remaining length of the chromosome. The striking differences in patterns of compensation in corn and cotton can rather easily be explained on the basis of patterns of chiasmata formation. The corn chromosomes studied have map lengths between 120 and 150 units. The cotton chromosome is slightly under 100 units long. If we assume that at least one chiasma regularly forms in each arm of the chromosome, then the long arms in the corn chromosomes usually have two chiasmata; cotton, only one. With two chiasmata available as in corn, the one nearest to the inversion and proximal to it would effect compensation. Diagrammatically, we could picture this as the reduction in standard deviation of the normal distribution of the chiasma frequency curve without change in total crossovers. The curve would be very steep with a narrow base. The distribution of the other chiasma, located nearer the centromere and farther removed from the inversion, would remain unchanged, as would the crossover frequencies resulting from it. On the other hand, in cotton, there is only one chiasma to handle the compensation. The base of its frequency curve would be shortened but the form little changed. Consequently, compensation would be spread rather evenly over the chromosome from one tail of the curve to the other.

If this is the correct interpretation, the failure of compensation in a segment distal to the inversion in corn suggests very strongly that chiasma formation is controlled by the centromere or by a specialized region immediately adjacent to it (Lima de Faria, 1954). An inversion interrupts the "channel of communication" between the centric region and the end of the chromosome—in effect, the proximal end of the inversion marks the limit of centric control. Similarly, the centromere itself constitutes a boundary between two separate systems of chiasmata organization (there is no interference across a centromere).

5. *Pseudo-alleles*

It may be a mere coincidence, but is worthy of note, that the approximate modal positions of chiasmata in the X-chromosome of Drosophila which are indicated by Weinstein's data suggest that a single chiasma usually forms in the *lozenge* region and that double chiasmata usually form in the *scute* and *white eye* regions, respectively. These three "complex loci" have

exercised the imagination of geneticists for a considerable period. One wonders if part of their "complexity" may be due to their locations at sites particularly vulnerable to recombination, and not entirely to any unique feature of size or structure which they may possess. It would be interesting to know if the transposition of pseudo-alleles to new sites (as might be achieved in a homozygous inversion) would transfer the pseudo-allelic phenomena to loci occupying their former position.

6. *The effect of temperature on crossing-over*

Plough (1917, 1921) demonstrated a striking effect both of abnormally high and abnormally low temperatures administered during oögenesis on crossing-over in Drosophila. The abnormal temperature increased crossing-over in the centric regions of Chromosomes 2 and 3 and had little effect on median regions. No effect was observed in the X-chromosome, but it is clear from Plough's data that the near centric region was unmarked in his stocks. His data can be interpreted satisfactorily in the same manner as was suggested in Section 3 for the Schultz-Redfield effect, namely, a change in modal position of chiasmata with increase in chiasma frequency.

Of particular interest was Plough's quantitative analysis of the temperature effect. Temperature changes within the range of approximately 20°–27° C had little effect; above and below these limits, there was a sharp rise in crossing-over (that is, in the proportion of double chiasmata). Plough (1917) made the acute observation that the form of the dose/effect curve was not that expected on the basis of "an ordinary chemical reaction following Van't Hoff's law" but followed almost exactly in form and range of temperatures the dose/effect curve found in muscle contractions. He speculated that the effect might correspond to a change in colloidal state.

It seems probable that the temperature effects which resulted in a change in chiasmata pattern in Plough's experiments may be causally related to the cold treatment effects observed by Darlington and La Cour (1940) on Trillium chromosomes. Regions, which under normal temperature conditions appear to be precociously charged with DNA during interphase, failed to develop DNA after cold shock. Further, such "starved" regions were greatly attenuated throughout the division cycle and failed to disjoin normally at anaphase. This might suggest that replication had failed in these regions. The *time* of replication and the *pattern* of recombination appear to be associated, and it may well be that a delay in DNA synthesis alters the position of chiasmata and only incidentally increases their frequency.

SUMMARY

From a re-examination of Weinstein's forty-year old data on recombination along the total length of the X-chromosome in *Drosophila melanogaster*, it is concluded that the number of chiasmata is greatly restricted. Over 90 per cent of the recombination was attributable to the effects of single chiasma bivalents and less than 0.5 per cent to bivalents with triple chiasmata. Each type of bivalent produced chiasmata in rather definite modal positions.

A single chiasma was usually located in the mid-region of the chromosome. When double or triple chiasmata were formed, the proximal one was located closer to the centromere, and the others were crowded toward the distal end of the chromosome. It is pointed out that this pattern of chiasma location may have rather far-reaching consequences on genetic theory and, in particular, on interpretations of interference, relative map lengths, compensatory crossing-over both between and within chromosomes, temperature effects, and the apparent complexity of pseudo-alleles.

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THE EDGE EFFECT IN POPULATION GENETICS

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Interest in the study of peripheral populations has arisen in several different branches of biology. At first it was a systematic problem, when it was noted that there were often distinct forms living at the edges of the ranges of species. Among European examples may be mentioned microtine rodents (Hinton, 1926), hares (Matthews, 1952), bumble bees (Richards, 1935), the European robin (Lack, 1953) and, at a lower level of distinctness, the meadow brown butterfly (McWhirter, 1957). A similar phenomenon has been recorded among birds in New Guinea (Mayr, 1954), and in South East Asia (Mayr and Vaurie, 1948). From other animal groups Brown (1957) has cited the distribution of forms of the frog *Rana pipiens*, as determined by the work of Moore, and of the lizard *Amphisbaena fuliginosa*, as described by Vanzolini.

In Europe it is evident that climatic changes since the end of the Pliocene have had an effect in determining the nature of the present-day fauna. The appearance and distribution of peripheral forms have been put down to alteration in habitat associated with successive changes in glaciation and isostatic and eustatic movements (Beirne, 1943; Hinton, 1926). According to this argument, peripheral forms are relicts which have been isolated from their relatives, and, because of subsequent changes in geography, have not been ousted by the newer species or forms established in habitats they used to occupy elsewhere. Two factors are being considered; on the one hand the geographical distribution of the different forms of a species and on the other, past events which could account for the distribution. Of course, in suggesting that there is a causal relation, one also considers implicitly what is known about the origin of evolutionary change in the species discussed. In this field there is sufficient new evidence for the whole question to merit re-examination (Mayr, 1947, 1954). It is evident that the genetic responses of animals to their environment, which are at the root of the observed differences, may depend to a large extent on the structure of the breeding group to which they belong, especially on its effective size and the number and range of habitats which it occupies. (Different aspects are considered in Dobzhansky, 1957; Wright, 1931, 1959). Some work, especially that which deals with the effects of gene flow on the ability of a population to respond to selection (Wright, 1938, 1946) and the circumstances which facilitate the development of a coadapted genetic system (Brncic, 1954; Dobzhansky, 1950; Mayr, 1954), indicates that the conditions

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experienced by peripheral populations could in fact be most favorable to rapid evolutionary change. In that case, far from being relicts, the morphologically different types could be of very recent origin. There is not complete agreement about the relative importance of the various contributory factors, however [for instance, Thoday (1958) minimizes the importance of the supposed reduction of gene flow], and the general theory of interdemic selection developed by Wright can also be taken as support for the relict hypothesis (Brown, 1957), so that it is necessary to define carefully the factors supposed to be involved and to weigh their importance.

The relict hypothesis was originally a probable inference from the available data. The ways in which its correctness could be assessed or substantiated were limited because of the lack of information about the causes of diversity. A close examination of peripheral populations may help to establish the relative importance of the different factors which have recently been postulated and, in doing so, would not only clarify the question of the origin of edge forms, but also provide information of more general value to the study of evolutionary change.

Examples

Some of the classical examples of peripherally divergent populations come from the European small mammal fauna. The subspecies of the bank vole (genus Clethrionomys) have often been mentioned (Richards, 1935; Brown, 1957). Although they have been quoted as a good example of relict distribution, there has recently been some doubt cast on this interpretation (Steven, 1953; Delany and Bishop, 1960), so that it will serve as a useful illustration in the present context.

The bank vole is divided into a number of forms which have been called variously species and subspecies. Hinton (1926) puts them in four species groups, of which the relevant ones are the *Clethrionomys glareolus* and *C. nageri* groups. The former are, on the whole, smaller and have smaller cheek teeth, less powerful jaw musculature and a less robust skull than *nageri*. The last upper molar is not as frequently of the type known as complex. The coat color is usually darker in *nageri* than in *glareolus*.

Members of the *nageri* group have been regarded as less successful than *glareolus* when the two come into competition. The argument to this effect in Barrett-Hamilton and Hinton (1914) and Hinton (1926) is well summarized by Harrison Matthews (1952). Of the two "the *nageri* group appears to be less vigorous than the *glareolus* group, for wherever a subspecies of the *glareolus* group invades a territory occupied by one of the *nageri* group it replaces it. This conclusion is arrived at by a consideration of the present geographical distribution of the subspecies, and of the fossil remains showing their former distribution. It is probable that most of Europe was once inhabited by members of the *nageri* group, which spread westwards from a centre in the east, arriving in Great Britain after the separation of Ireland

so that it was unable to reach that country. *C. g. glareolus* arrived from the east at a later date, and though a smaller subspecies, replaced *nageri* over the greater part of its range, leaving the remnants of that subspecies only in Scandinavia, on mountain tops, or isolated islands."

This statement is based on the presence of three members of the *nageri* group on islands off the west coast of Britain (Skomer, Mull and Raasay), and on others living in mountainous regions in west and central Europe and found as fossils in British Pleistocene deposits. Steven (1953) has examined the differences between the British island populations and *C. g. glareolus* from the mainland. He finds that the island animals are consistently larger in size and remain so after several generations of breeding in captivity. By crossing individuals of different types, he showed that the shade of coat color, and probably the different tooth pattern, are characters controlled by single genes. He quotes evidence that the same is true of coat color in continental *nageri*, in which dark is dominant to the lighter *glareolus* color. In Britain the Skomer animals are light while the other two groups are dark, and the Skomer color is dominant to the others. Regarding the fossil material, Hinton describes *Evotomys (= Cletbrionomys) kennardi* which comes from late Pleistocene deposits. He puts it with the *nageri* group because of the large size of the skull, the heavy cheek teeth and the high frequency of complex molar pattern. Hinton also discusses material from older Pleistocene deposits which is put with the *glareolus* group, and others (*C. barrisoni*) from the late Pleistocene, so that the two species groups were present in Britain contemporaneously, and there is a longer recorded history for *glareolus* than for *nageri*.

The division into two groups is based on three characters. One of them (coat color) differs between members of the *nageri* group. Another (complex molar pattern) varies in frequency between island populations, and in both *glareolus* and *nageri* it is a polymorphic condition presumably maintained by some balance of selective forces. These two characters are under unifactorial control and are of a type which might be subject to local selection pressures. The third, body size, is the only one in which, by definition, all *nageri* populations differ from all populations of *glareolus*, yet it too could be influenced by selection. The relict hypothesis requires that it be a genetically conservative character. Zimmermann (1950) discusses four other European rodents in which large forms have a peripheral, mountain and 'refugial' distribution. The recognition of ecogeographical rules (Bergmann's and Gloger's rules), which cover variation of the sort in question with changes in climate, suggests that another interpretation may be possible (see Snow, 1954; Timofeeff-Ressovsky, 1940). The subject is discussed critically by Mayr (1956). Conditions on islands and mountains could be such that large forms are at a selective advantage, while this is not so in other parts of the range.

Another very similar distribution is found in the snail *Cepaea nemoralis*. In this species, too, regions at the edge of the range, including mountains and islands, are inhabited by large forms which have a higher frequency than usual of a unifactorial character, white lip (Lamotte, 1951; Cook and Peake, 1960). The belief that these populations inhabit refuges receives support from the distribution of large fossil specimens, which are widespread throughout the present range of the small forms.

Although one large-shelled population may differ from a small-shelled one in a number of characters, examination of the peripherally distributed populations shows that the only feature they all have in common is their size, and even in this there is very great variation. The problem is the same as in Clethrionomys. If size is an evolutionarily conservative character, the peripheral members may have in common an old gene complex which includes large size. If, on the other hand, it could be the result of local selection, then there is no positive evidence for this point of view. The subsidiary characters may be directly selected in the environment which leads to large size, or they could be factors associated with a gene complex somewhat different from the normal one for the species.

These are the elements of the difference of opinion as to the evolutionary significance of peripheral populations. In what follows the features which are important to each argument will be described, and the evidence supporting them will be discussed, especially that concerned with the importance of genetic interactions.

Centrifugal speciation

Brown (1957), in a stimulating paper, has pointed out that since novel combinations are most likely to arise where locally adapted, partially isolated populations meet, they are the most fertile areas from an evolutionary viewpoint. For a number of reasons, these are less likely to be at the edge of the range of a species than at its center. Populations in peripheral localities tend on the whole to be more isolated from each other than central ones (Carson, 1955). The range of environmental tolerance is decreased (Darlington, 1959; Dobzhansky, 1957) and migration between them is predominantly from one direction. For the same reasons, gene flow will tend to be from the center outward (Haldane, 1956) carrying the genetic novelties with it. Consequently, Brown believes that in most species the peripheral forms exhibit primitive characters, not having received the more advanced traits which spread in successive waves from places where the species is best established. If during isolation a sterility barrier should develop, the situation culminates in the presence of a new and more vigorous species in the central parts while a primitive, relict species remains at the edge.

Peripheral diversity

In contrast, Mayr (1954) emphasizes the fact that gene flow may play an important part in limiting the opportunity of a population to adapt to local

conditions. He points out, quoting examples, that an island form may differ very greatly from the population living in a similar habitat on the mainland. The latter, however, may be indistinguishable from another mainland population living a great distance away under different ecological conditions. Gene flow could be a determining factor, inhibiting change where there is migration between populations and allowing it where they are isolated. Isolated populations in peripheral areas, particularly those including fringing islands, are likely to start with their establishment by a few founder-individuals. When this occurs, the gene pool is restricted in a more or less random manner. The future development of the population will involve not only genetic adaptation in a new environment, which may differ in the niches available, but also coadaptation of the gene pool as the population increases in size. As a result, a new population so established may differ greatly from the parental form. Part of the distinctness will be due to the fact that, because of the increased isolation, there is no longer a premium on genes which do well on a variety of genetic backgrounds. The isolated population will be relatively invariable genetically and likely to be unsuccessful, but the process of large-scale genetic reorganization coupled with a new environment will occasionally allow a population to occupy a niche where it can expand in numbers to build up its genetic variability. In this way an evolutionary novelty may be produced which can spread and eventually occupy much of the parental territory.

Mayr suggests that the formation of peripheral isolated populations in the way he describes could sometimes, though only rarely, lead to important evolutionary change. One may also ask whether this is the process by which most of the distinct forms arose. The difference in emphasis concerns chiefly the effect of gene flow on the ability of semi-isolated populations to adapt, and the effectiveness of isolation and reduction in numbers, as opposed to gene exchange, as agents furthering genetic divergence.

Drift, migration and selection

One of the possible causes of diversity between small populations is the tendency for the frequency of alleles at different loci to "drift" to fixation. Migration and selection both have an important effect on this. It has been shown by Wright (1931) that the tendency to chance fixation of an allele at a single locus is likely to be offset by rates of immigration that are greater than $\frac{1}{2N}$ (where N is the effective population size), irrespective of population size. Since this necessitates interchange of only one individual every second generation, the chance that drift will materially affect the distribution of gene frequencies in a semi-isolated population is very small. The conclusion that the effect of drift is negligible in the face of the selective pressures which are likely to be operating was clearly allowed for by Wright and has been further emphasized by Fisher and Ford (1947, 1950). Falconer

(1960) points out that, according to Wright's treatment, selection begins to be more important than random drift when the selection coefficient is of the order of magnitude of $\frac{1}{4N}$. From this it appears that selection has an effect of the same order of magnitude (and about twice as great) as migration. In all but the smallest populations even slight selection or migration will counteract the tendency to fixation by random drift.

It has been suggested (Mayr, 1947, 1954) that gene flow could swamp response to local selection so that differentiation occurs only in isolated localities, such as islands, although local conditions on the nearby mainland may favor the same change. It is difficult to get a quantitative idea of the ability of migration to neutralize selective pressures, but by making some assumptions estimates can be provided in special cases.

The action of the selection tends to move gene frequencies towards an equilibrium point, and immigration from a population with a different gene frequency will cause a different level to be approached. In order to investigate the effectiveness of the factors determining the latter, a hypothetical model has been developed based on Steven's data for the dominant character, *complex*, in Clethrionomys. Calculations are made on the *assumption* that a single gene is involved.

In three subspecies, the frequency of this character is:

Subspecies	Location	Frequency
britannicus	mainland Britain	0.26
alstoni	Mull	0.28
erica	Raasay	0.75

Suppose that the gene for *complex* is at equilibrium frequency on Raasay, and that it is tending to the same equilibrium on Mull but is prevented from reaching it by immigration from the mainland. If the frequencies of genes, A and a, on Mull are p_1 and q_1, and the relative reproductive powers of the zygotes, AA, Aa and aa, are in the ratio $(1 - s) : 1 : (1 - t)$, then in the absence of immigration the frequency of A in the next generation is:

$$\frac{(1 - s)p_1^2 + p_1q_1}{(1 - s)p_1^2 + 2p_1q_1 + (1 - t)q_1^2}$$

If a fraction, k, of the population are immigrants from a population where the frequencies of A and a are p_2 and q_2, then in the next generation the frequency, p_1', of p_1 is given by:

$$p_1' = (1 - k)\left[\frac{(1 - s)p_1^2 + p_1q_1}{(1 - s)p_1^2 + 2p_1q_1 + (1 - t)q_1^2}\right] + kp_2$$

When $p_1' = p_1$ immigration is counteracting the tendency to advance to the equilibrium determined by s and t. By inserting numerical values for the other symbols, we may calculate the value of k required under different

selection pressures for this condition to be maintained. Then $k/(1-k)$ is the number of immigrants required per indigenous member of the population in order to prevent change.

Assume first that the gene frequency on Raasay is maintained by heterozygote advantage so that $p = 0.5 = q$. The selective pressures against the homozygotes will then be equal. On Mull, $p_1^2 + 2p_1q_1 = 0.28$, so that $p_1 = 0.1515$, and $q_1 = 0.8485$. Similarly, $p_2 = 0.1398$. Putting these values in the equation we obtain the following results:

$(1-t)$	$k/(1-k)$
.999	0.008
.990	0.077
.950	0.397
.800	1.785
.600	4.348
.500	6.250

Values of s greater than 0.01 would require immigration in excess of about eight per cent of the population size for change to be prevented under the prescribed conditions. If we assume that the observed frequency of *complex* on the isolated island is not a stable one but that it is proceeding to fixation, then for the value of $(1-t) = 0.990$, $k/(1-k) = 0.111$. Under these conditions the number of immigrants required is of the same order, but rather higher.

It is probable that high selection pressures are relatively common. Approximately 30 per cent selective advantage has been observed in the spread of a melanic form of the moth *Biston betularia* (Kettlewell, 1958), admittedly due to a man-made change. Allison (1956) gives a value of about 20 per cent for the sickle-cell heterozygote, and Dobzhansky (1956) records a change in the frequency of a chromosome type in *Drosophila pseudoobscura* under natural conditions which indicates a selective advantage that is probably of this order. Similar values have been demonstrated in Paratettix (Fisher, 1939), in Cepaea (Cain and Sheppard, 1954) and in Maniola (Dowdeswell, Ford and McWhirter, 1960; Dowdeswell, 1961). Thoday (1958) states that, when he subjected subpopulations in an artificial selection experiment to five per cent selection in opposite directions, even 50 per cent gene flow did not prevent divergence (see also, Millicent and Thoday, 1960). Certainly, in the face of larger selection coefficients in nature the swamping effect of gene flow can be of little consequence.

When there is strong selection it would not be surprising if isolated populations were found to undergo rapid change. The St. Kilda house mouse and the Icelandic long-tailed field mouse may be mentioned as examples which have reached at least subspecific distinctness in a few hundred years (Steven, 1953). E. C. Zimmerman (1960) suggests that a complex of five or more species of moths may have evolved in Hawaii in the space of a thousand years. These examples indicate the rates which may be attained.

The process of change is facilitated by the alteration of the ecological niche which is frequently associated with a peripheral location (Brown and Wilson, 1956). The examples discussed by Zimmerman involve specialization on a new food-plant; which is just one aspect of the change and restriction in habitat which may be encountered. Differences in ecology between British and Continental members of a species are seen in the Opilionid *Nelima sylvatica* (Bristow, 1935), the moths *Malacosoma castrensis* and *Lymantria dispar* (Ford, 1955) and the grasshoppers *Gomphocerippus rufus* and *Stenobothrus lineatus* (Richards and Waloff, 1954). The first two of these occupy coastal habitats in Britain but have a general inland distribution on the Continent. Many examples of such a distribution are given by Chapman (1899).

Remembering that k is that fraction of the population consisting of immigrants from a neighboring population of different gene frequency, we see that very high values of k *could* be encountered under peripheral conditions and could counteract the effects of selection. Obligate outcrossing between two equal-sized populations would be equivalent to a value of k equal to 0.5, which is the maximum for interbreeding, and Thoday (1958) concludes that immigration will fall below this level in the wild. However, it is possible for many more individuals to move from a large population to a small one than is indicated by this figure, and they will affect the frequency in future generations. In an example quoted by Birch (1960), where populations of a moth (*Heliothis armigera*) are maintained on the northern edge of their range by emigration from the south, k must come close to 1. Local adaptation under these conditions would be impossible. If the behavior of all individuals was the same, the value of k would be a function of the ratio of the two population sizes (numbers and density), subject to restriction by the number of immigrants which the small population is capable of receiving. Differential behavior, leading to non-normal dispersal patterns, further complicates the picture in practice.

One may conclude that where migration between populations is of the order of one or a few per cent, as it must commonly be, immigration can be discounted as a factor determining gene frequencies at a single locus; but where marginal populations continue to exist, despite reduced reproductive capacity, as a result of immigration, the effect on gene frequency will be important. It is possible, however, that this greatly underestimates the importance of migration because of its effect on coadaptation of the gene complex.

Coadaptation

It has become customary for writers on population genetics to emphasize the fact that natural selection concerns individuals rather than genes, so that its results are influenced by interactions between the components of the gene pool. This is clearly apparent when the end products are associ-

ated morphological structures; it is observed also in studies of the gene arrangement itself. Examples of coadaptation include interaction between homozygous loci, selection for heterozygosity at many loci, with accompanying development of mechanisms to prevent recombination, and the phenomena spoken of by Dobzhansky (1955) as organization effects. Diverse examples are given by Reeve and Robertson (1953), Thoday (1960), Vetukhiv (1954), Wallace (1953), Wallace and Vetukhiv (1955), Brncic (1961), etc.

The loss of fitness in the F_2 of interpopulation crosses, after heterosis in the F_1, is a result ascribed to the effects of recombination on such coadapted systems (King, 1955; Vetukhiv and Beardmore, 1959). It is of interest here because it is possible that the presence of immigrants may be felt more because they tend to break up, or prevent development of, an integrated genetic system than because they themselves are ill-adapted to the local conditions. The authors mentioned worked with characters under multifactorial control, but the argument also applies to genes with large effects when their adaptive value depends on a system of modifiers.

To take a particular case, Williamson (1957) has pointed out that, when a polymorphism is maintained by heterozygote advantage, any alteration of the phenotypes which tends to equalize the selective coefficients of the homozygotes will raise the proportion of heterozygotes and may itself be selected. One way in which the change could be effected would be for the heterozygote and the less favored homozygote to become more alike through modification of dominance. It is shown above that, given certain parameters which include the selection coefficients, one can predict the equilibrium frequency resulting from a given amount of immigration. If the presence of immigrants serves to break down the modifiers which determine appearance, the selective coefficients will be altered and the predicted equilibrium will not be reached. The importance of this result depends on the relative change in the fitness of the population (however fitness be defined).

When the frequency of highly adapted genes is maintained at different levels in two populations because of environmental differences, their respective gene pools will tend to become unlike. Migration between them will reduce the overall fitness of each, but because the offspring of interpopulation crosses will be the least fit, there is a chance that reproductive isolation will be built up. This would be an advantageous development if the direction of selection remained constant: its importance in nature depends on the size and stability of the selective coefficients experienced. Short-term fluctuations in both direction and intensity will cause fluctuations in the adaptive value of the respective combinations. Experimental results which show how variable the fitness of a genotype may be from time to time with slight environmental differences are discussed by Wallace (1959). Under natural conditions in peripheral habitats much greater variation may be expected, and the disadvantage of destruction of coadaptation by immigrants may be offset by the usefulness of the genes which they bring with them.

CONCLUSION

Mayr (1959) suggests that the groups where evolutionary progress is most likely to occur are those which have 'universal' members with much heterozygosity and concealed variability, and also members that can become closely adapted to local environments. He regards the latter as foci for evolutionary change. The argument is taken further by Carson (1959), who suggests that, as a result of selection for heterozygous combinations, the universal populations become unable to proliferate new forms, so that the evolutionary role is necessarily left to the small peripheral units. However, improvement in a large population through increased heterotic buffering can also be regarded as evolutionary change, and it may have more far-reaching effects than changes in marginal isolates.

When examining particular examples of peripherally distinct forms, we may expect to find the results of both processes. In some species, advanced features spread from areas where they are most successfully established. In others, small isolated populations in marginal habitats become differentiated from the main body of the species. Here I would include examples of the type discussed above, which have been thought of as inhabiting refuges. There is insufficient evidence, both for their affinities with each other and against the possibility of rapid change in similar directions, to support the belief that they are relicts; differentiation under similar selection pressures appears more probable. But whether they are often the starting point for new evolutionary developments is less certain. To determine the relative contributions made by widely-adapted and locally-adapted populations, it is necessary to know more about the parts played by coadaptation and by gene exchange in advancing or retarding change.

SUMMARY

Many animal species have been observed in which populations living near the edge of the range differ from those more centrally placed. Sometimes different peripheral populations resemble each other more closely than they do the central ones. Such a distribution must be due either to more rapid change in central than in peripheral localities, or *vice versa*. In discussion of particular instances each alternative has its adherents. It is suggested here that in two examples from the European fauna (bank voles of the genus Clethrionomys and the snail *Cepaea nemoralis*) the distinct edge populations are less likely to be relicts than recent developments. Consideration of the response of populations of different sizes and breeding structure to the environmental conditions in various parts of the range suggests that we shall not know whether this is usually so, or whether peripheral populations play an important part in evolution, until the effects of coadaptation and interpopulation migration are better understood.

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EVIDENCE FOR PARTHENOGENESIS IN *ASPERGILLUS NIDULANS*

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Pontecorvo and Roper (1953) observed asci with either eight or 16 ascospores in perithecia from diploid strains of *Aspergillus nidulans* (Eidam) Winter; the frequency of each type of ascus depended on the strain. Although a systematic genetic analysis of the ascospores was not attempted, a random sample from one heterozygous diploid strain indicated that the spores were segregating for the markers for which the strain was supposed to be heterozygous. According to Pontecorvo and Roper, "If the asci arise parthenogenetically in a diploid heterozygous for known markers, these asci should show ordinary diplohaploid segregation; if they arise from patches of mycelium which have reverted to the haploid condition, usually they should not segregate at all." Elliott (1960) concluded from a cytological study of a diploid strain that the ascospores were derived from a diploid nucleus, that is, were parthenogenetic.

In the present study, a genetic analysis of a random sample of ascospores from a diploid strain of *A. nidulans* was undertaken to obtain additional evidence of parthenogenesis in this species.

PROCEDURES

The general techniques used throughout this investigation were the same as those described by Pontecorvo (1953). Different minimal (Garber, Wyttenbach, and Dhillon, 1961) and complex media were used. The latter medium contained 0.5 per cent yeast extract, two per cent glucose, and two per cent agar.

A diploid strain was synthesized using methods described by Pontecorvo (1953) from haploid strains 182 and 183 supplied by Dr. R. W. Barratt. Strain 182 is yellow (*y*), acriflavine-sensitive (*acr*), and requires para-aminobenzoic acid (*pab*) and biotin (*bio*); strain 183 is white (*w*), acriflavine-resistant (*Acr*), and requires methionine (*met*) and pyridoxine (*pdx*). White is epistatic to yellow. The diploid strain was light green, acriflavine-resistant, and prototrophic, growing on the minimal medium. The genotype and the position of the centromere in each of the three linkage groups (Pontecorvo, 1958) are presented in figure 1.

To remove conidia, mature perithecia were rolled on the surface of plates containing four per cent agar until they were shiny black. Although it was possible that a few conidia may have remained, they were not a serious source of error in scoring colonies from ascospores. To obtain ascospores,

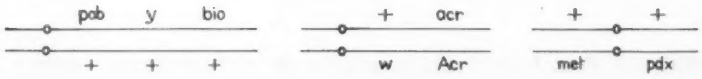

FIGURE 1. Genotype and position of the centromere in each of the three linkage groups in a diploid strain synthesized from haploid strains 182 and 183 of *Aspergillus nidulans*.

perithecia were crushed against the wall of a small test tube containing either 0.2 or 1.0 ml sterile distilled water. Since only two to four per cent of the ascospores germinated, the contents of several perithecia from one diploid colony were pooled. Appropriate dilutions of the suspension of ascospores were plated on complex medium to obtain isolated colonies which were then randomly picked before they had developed color.

RESULTS

Of the 792 colonies obtained from ascospores, 741 were green, 11 yellow, and 40 white. The green colonies were either light (731) or dark (ten) green. None of the yellow, white, or dark green colonies sectored; 724 light green colonies displayed white or yellow sectors and seven, white, yellow, or dark green sectors.

One hundred four randomly selected light green colonies, 38 white, 11 yellow, and nine dark green colonies (table 1), and each of the seven dark green sectors on light green colonies were tested for the resistance or sensitivity to acriflavine and for their nutritional requirements. The light green colonies were acriflavine-resistant and prototrophic; the other colonies and the sectors included recombinants. The ratio of mutant to non-mutant for the five markers in the white, yellow, and dark green colonies was determined: *Acr/acr*−41/17, *pab*/+−16/42, *bio*/+−20/38, *met*/+−25/33, *pdx*/+−31/27.

Perithecia from haploid strains 182 and 183 gave a large number of germinating ascospores but perithecia from the diploid strain gave only a low number. The yield of germinating ascospores from perithecia of light green, dark green, white, and yellow colonies obtained from ascospores of the diploid strain was determined. A suspension of the contents (ascospores) of one mature perithecium from each colony was plated on complex medium. Perithecia from 39 light green colonies and from all yellow colonies gave a low yield of germinating ascospores; ten of 39 white colonies also gave a low number. Four of the ten dark green colonies did not have any detectable perithecia; perithecia from two colonies gave a low and from four colonies, a high yield of germinating ascospores. Consequently, presumably haploid and known diploid colonies could not be distinguished on the basis of yield of germinating ascospores.

The resistance or sensitivity to acriflavine and the nutritional requirements of the colonies giving perithecia with a high yield of germinating

TABLE 1

Color, resistance (*Acr*) or sensitivity (*acr*) to acriflavine, and nutritional requirements of colonies from ascospores of perithecia of a diploid strain synthesized from haploid strains 182 and 183 of *Aspergillus nidulans*.

Nutritional requirements	Number of colonies			
	Color			
	Light green	White	Yellow	Dark green
Acr prototrophic	104	17	0	1
Acr pdx met pab bio	0	3	2	0
Acr pdx pab bio	0	2	0	0
Acr pdx bio	0	2	1	0
Acr pdx met	0	2	0	0
Acr pdx pab	0	1	0	0
Acr pdx	0	6	0	0
Acr bio	0	2	0	0
Acr met	0	2	0	0
acr prototrophic	0	0	0	1
acr pdx met pab bio	0	0	6	1
acr met pab bio	0	0	0	1
acr pdx met	0	1	0	4
acr met	0	0	2	1
Total	104	38	11	9

ascospores were determined. It was not possible to find a common genotype for these colonies; that is, recombinants of all types were present.

Colonies from ascospores of one perithecium from each of 38 light green colonies were scored for color. Ascospores from 18 perithecia gave only light green colonies; ascospores from 12 perithecia gave dark green, yellow, and white colonies and from eight perithecia, light green, dark green, yellow, and white colonies.

DISCUSSION

Since Elliott (1960) found no cytological evidence for a tetraploid in the ascus primordium of a diploid strain, he concluded that the ascospores had been derived from a diploid nucleus. Furthermore, other evidence indicated that the ascospores were haploid and presumably the products of meiosis. In our diploid strain, however, approximately 94 per cent of the ascospores were diploid. This observation suggested that the majority of the ascospores were derived from the initial diploid nucleus by mitosis and only a few by meiosis.

The diploid light green colonies were heterozygous for white and yellow; the dark green colonies were either diploid and homozygous for the nonmutant alleles or haploid and hemizygous for these alleles. The difference in color probably represented a dosage effect in the diploid strain. Since

the dark green colonies included recombinants for the five markers, they were probably haploids.

Since white was epistatic to yellow, the genotype of the white colonies from ascospores of the diploid strain may have been either *w+* or *wy*. Assuming that 50 per cent of the white colonies had the chromosome carrying *pab y bio*, the frequency of *pab* and/or *bio* colonies would have been expected to be significantly greater than that of colonies with either or both non-mutant alleles. The ratio of mutant to non-mutant colonies supported this prediction. Since *met* and *pdx* were on a third chromosome, the frequency of mutant to non-mutant colonies was expected to be approximately equal, and the data supported this expectation.

Although the haploid strains used to synthesize the diploid strain gave a large number of germinating ascospores, the presumably haploid segregants from perithecia of the diploid strain often gave a low number of germinating ascospores. Furthermore, four colonies did not yield any detectable perithecia. No explanation for these observations is available at this time.

According to Pontecorvo and Roper (1953) and to Elliott (1960), ascospores from perithecia of diploid strains were haploid products of meiosis. The ascospores from perithecia of the diploid strain in our investigation were either diploid products of mitosis or haploid products of meiosis. In one perithecium, it was possible to obtain only diploid or only haploid ascospores. Since eight perithecia gave both haploid and diploid colonies, their ascospores may have been the products of meiosis or mitosis in different asci of the same perithecium. It is also possible that the diploid colonies came from conidial contamination. It cannot be stated at this time that all perithecia from one diploid colony followed the same pattern with respect to the type of ascospores which were produced.

SUMMARY

Colonies from ascospores of perithecia from a diploid strain of *Aspergillus nidulans*, synthesized from haploid strains 182 and 183, were either diploid or were recombinants and presumably haploid; the former constituted approximately 94 per cent of the colonies. Although perithecia from diploid colonies contained a low number of germinating ascospores, perithecia from recombinants had either a high or a low number of such ascospores and four presumably haploid colonies did not have any detectable perithecia. It was possible to obtain only diploid, only haploid, or both diploid and haploid ascospores from a perithecium.

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THE ROLE OF CHROMOSOMAL TRANSLOCATIONS IN URODELE EVOLUTION AND SPECIATION IN THE LIGHT OF WORK ON GRASSHOPPERS

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It has been reported by Callan and Spurway (1951) that multivalent associations (chains of three and four metacentric chromosomes) occur at meiosis in F_1 interracial hybrids of the urodele *Triturus cristatus*. They conclude that the subspecies *karelinii* differs from the subspecies *carnifex* by at least one translocation and from the subspecies *cristatus* by at least two. The same authors (Spurway and Callan, 1960) have reported a similar cytological picture in the spermatogenesis of F_1 hybrids between *Triturus vulgaris* and *T. belveticus* and have interpreted it in the same manner. Lantz and Callan (1954) observed multivalent formation in backcross hybrids from F_1 female (*T. marmoratus* × *T. c. karelinii*) × male *T. marmoratus* and concluded that these species differ by at least four translocations which possibly form a serial system.

It is clear that a fairly uniform and consistent pattern emerges from all this work and that there can be no dispute as to the facts. Various interpretations are, however, possible. Lantz and Callan (1954) state: "It may be that translocations are merely incidental concomitants of race and species divergence in the course of evolution. Taking the opposite view, it is possible to argue that translocations have been critical events which permitted race and species adaptive divergence to occur." It is the latter possibility which renders the cytological observations on Triturus hybrids of considerable general significance.

Wright (1941) has pointed out the very strong selection pressure which must in general exist against the establishment of reciprocal translocations in natural populations unless these are very small. This selection is due to the reduced fertility of the heterozygotes which will form a certain number of aneuploid gametes unless they have a type of meiosis in which the translocation chromosomes invariably participate in the formation of multivalents which orientate disjunctionally on the meiotic spindle (either bivalent formation or non-disjunctional orientations of multivalents will lead to a reduction of fertility).

Spurway (1953) has argued that many peripheral Triturus populations are, in fact, sufficiently small for translocations to reach fixation by drift and has drawn a graphic picture of an advancing species population consisting of small isolated colonies crossing hundreds of ecological barriers; at each there would be a chance for a translocation to establish itself. Once homozygous, a translocation might be expected to act as an isolating mechanism

and might tend to prevent the breakdown of coadapted gene complexes by immigrants from other populations.

Lantz and Callan have put forward an alternative hypothesis according to which newly-arisen translocation heterozygotes might in fact show close to 100 per cent disjunctional multivalents at meiosis.

An entirely different possibility seems to exist, however. It is conceivable that both the parent species or races of these Triturus hybrids may be homozygous for several minute reduplicated segments at the ends of their chromosomes so that they would be tetrasomic, hexasomic...etc., for the same segments. If this were so, racially or specifically pure individuals would be theoretically capable of forming multivalents but might not do so in practice if the reduplicated regions were sufficiently minute by comparison with those represented twice in the diploid nucleus. On the other hand, in hybrid material with considerably reduced synaptic affinity there might be a chance for "anomalous" pairing between terminal segments of otherwise nonhomologous chromosomes to occur and for chiasmata to be formed in such regions.

This possibility that the hybrid salamanders studied by Callan, Spurway and Lantz may be homozygotes for duplicated (tetrasomic, hexasomic, or even octasomic) regions at the chromosome ends rather than translocation-heterozygotes is suggested by two kinds of observations on the cytology of certain grasshoppers.

(1) In individual grasshoppers which are double or multiple heterozygotes for pericentric inversions (that is, in which several chromosome pairs are each represented by an acrocentric and metacentric element), it *occasionally* happens that a quadrivalent is formed between two structurally heterozygous pairs. In all cases hitherto observed it is the two acrocentric elements which are associated end to end. In figure 1 we illustrate an instance in *Trimerotropis gracilis*; exactly comparable cases have been observed in individuals of *Moraba scurra* heterozygous for the Standard/Blundell and Standard/Tidbinbilla sequences (White, 1957). In both these species such anomalous pairing is very rare, not more than one in 10,000 first metaphases showing it; but in *Austroicetes interioris* (White and Key, 1957) anomalous associations are considerably commoner. In all three species they are entirely clear and unmistakable when they do occur, and it is evident that we are dealing with a characteristic cytogenetic phenomenon. Since the association is always a terminal one, no cross-shaped configuration can be observed but there can be no reasonable doubt that we are dealing with terminalized chiasmata.

True synapsis is apparently suppressed between mutually inverted chromosome segments in these grasshopper bivalents so that no "reversed loops" are present at pachytene; instead, a kind of nonhomologous pseudosynapsis occurs. It is presumably the suppression of "true" synapsis which permits or facilitates the occasional pairing between minute regions at the ends of otherwise nonhomologous chromosomes. Such pairing does, however, indicate that these minute regions are, in fact, homologous. In the cases

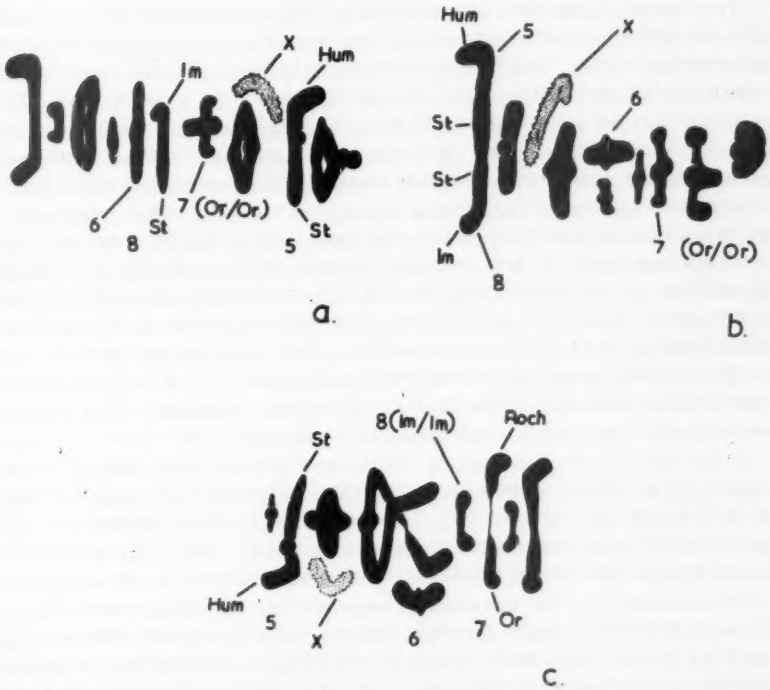

FIGURE 1. Three first metaphases of the grasshopper *Trimerotropis gracilis* (Limerick Canyon, Humboldt Range, Nevada). *a* and *b* are from the same individual, which was structurally heterozygous for chromosome pairs 5 (Standard/Humboldt) and 8 (Standard/Imlay) and homozygous for chromosome 7 (Oreana/Oreana). In *b* the Standard 5 and Standard 8 chromosomes are paired end-to-end. Cell *c* is from another individual, which was heterozygous for a reciprocal translocation between two of the larger chromosomes and shows a chain of four (some other cells showed a ring of four); this individual had the constitution Standard/Humboldt, Oreana/Rochester, Imlay/Imlay.

cited we are probably dealing with duplication of minute regions in the short arms of acrocentric chromosome elements—possibly the whole of the short arm, but at any rate its telomeric end.

It should be emphasized that this kind of anomalous pairing is very likely to lead to non-disjunctional first anaphases. In species with multiple inversion-heterozygosity natural selection against the occurrence of such behavior will consequently have been extremely strong, and it is not surprising that it should be very rare at the present time. One might expect that anomalous pairing of this kind might be much more frequent in the case of newly arisen pericentric inversions or in heterozygotes obtained by hybridizing individuals from different, structurally monomorphic geographic populations.

(2) A rather similar kind of anomalous pairing was observed in F_1 hybrids between individuals of *Moraba scurra* collected at localities about 150 miles apart (White, 1957). But in these F_1 hybrids such anomalous associations were exceedingly common; they involved at least eight of the total number of 32 chromosome ends in the diploid set, and they were not necessarily associated with heterozygosity for pericentric inversions. These anomalous associations occurred in individuals whose chiasma frequenc: was significantly *less* than that of individuals captured in the wild. Thus, once again we have a combination of suppression of normal chiasmata with the presence of anomalous ones. A few multivalents were also observed in F_1 hybrids between the grasshoppers *Chorthippus bicolor* and *Ch. biguttulus* (Kingstedt, 1939) and in those between *Trimerotropis suffusa* and *Circotettix verruculatus* (Helwig, 1955). The occasional terminal associations between two bivalents in the tettigoniid *Metrioptera brachyptera* studied by White (1940) are probably also due to duplication of terminal segments rather than to translocation heterozygosity as originally supposed.

If the multivalents observed by Callan and Spurway were indeed heterozygous for reciprocal translocations, we should expect a fair number of rings as well as chains. In fact, it is only in the single hybrid between *T. vulgaris* and *T. belveticus* that such rings were seen. Both rings and chains would be expected on the hypothesis of duplicated ends. Lantz and Callan (1954) assumed that the translocated segments are very short terminal ones because individuals which form multivalents are also capable of forming as many as ten bivalents with chiasmata in both arms. But on the interpretation favored by Callan and Spurway, it is not at all clear why they should be short; in fact, one would expect that reciprocal translocations of long regions, that is, nearly entire chromosome arms, would be more likely to show regular multivalent-formation at meiosis.

The presence of terminal regions of chromosomes in the tetrasomic, hexasomic, octasomic . . . condition implies that translocations have occurred, but does not specify *when* they occurred. Presumably most of them were reciprocal translocations of extremely minute segments. In the case of many such translocations the regions interchanged would be so small that the deficiency-duplication homozygotes (that is, individuals homozygous for one of the original chromosomes and one of the translocated ones) would probably be viable, as seems to have been the case in the peculiar mantid *Rhodomantis pulchella* (White, 1961). Such a situation is likely to lead to extensive homologies between the tips of many otherwise nonhomologous chromosomes, provided that the mechanism of meiosis is such that multivalents are not normally formed. It may be suggested that such conditions occur in many species of animals and that it is only when synapsis is largely suppressed or inhibited (as it is in multiple heterozygotes for pericentric inversions and in interpopulation, interracial or interspecific hybrids) that an opportunity occurs for anomalous synaptic unions to take place, with occasional chiasma formation in these minute terminal segments. The multivalents formed in the interpopulation hybrids of *Moraba scurra* simulate the configurations

which would result from heterozygosity for translocations, but there are too many different kinds of alternative configurations involving the AB, CD, EF and at least one of the smaller chromosomes (White, 1957)—alternatives which all coexist in the same individual. The identification of these alternative multivalents is made possible by the fact that all the chromosomes concerned are easily distinguishable; if they were all the same size, we should not recognize the different associations of chromosome ends as such.

On general grounds, since loss or duplication of minute chromosome regions is not invariably lethal, exchange of chromosome tips (that is, telomeres plus one, two or three genetic loci) with their consequent reduplication in tetrasomic, hexasomic, etc. form is probably one of the commonest processes in chromosomal evolution. It is not a particularly conspicuous one, in the sense of giving rise to obvious cytological changes. Neither is it likely to have been of any great importance in speciation and evolution. It is, in fact, only under the special circumstances of suppressed or interrupted synapsis which occur in hybrids or inversion heterozygotes that we can expect to have direct evidence of it.

Both Callan and Spurway's interpretation and the one presented here assume that translocations *have* occurred in Triturus. But on the hypothesis of the duplicated ends, such translocations might have arisen at any time in the past—even in the very remote ancestry of the genus—without reference to racial or specific differentiation.

It will not be easy to obtain crucial evidence as to whether Callan and Spurway's interpretation of the Triturus hybrid material or the one put forward here is correct. Conceivably, but improbably, both types of rearrangements might be involved. If chromosome arms could be individually identified in meiotic metaphases, it should be possible to decide between the two alternatives, since on the Callan-Spurway interpretation associations of chromosome ends should be invariable (each end being only capable of association with one other end, in a diploid nucleus) while on the "hypothesis of duplicated ends" there might be alternative associations of chromosome ends. Even without identification of chromosome arms, coexistence of nuclei with 23 or 24 strictly terminal chiasmata in the same individual as cells with chains of four, if it could be demonstrated, would be evidence in favor of "duplicated ends." Evidence of this kind may, in fact, exist in the case of Lantz and Callan's hybrid male 142. Their figure 5a shows 18 terminal chiasmata in bivalents, whereas 16 would be the maximum number possible on their hypothesis if this individual was, as they suggest, capable of forming two chains of six chromosomes at meiosis. But this "proof" depends on the chiasmata in the bivalents being strictly terminalized, rather than slightly subterminal and this is impossible to determine. It also depends on the generally-accepted view that chiasma-terminalization is arrested by changes of homology, which may not be universally true.

A very detailed study of the so-called lampbrush chromosomes of oöcyte nuclei of four subspecies of *Triturus cristatus* (Callan and Lloyd, 1960) has not provided any definite evidence bearing on the alternative interpretations

of multivalents in interracial hybrids, but further investigations, particularly on lampbrush chromosomes of hybrid individuals, might very well do so.

Obviously, nothing in the above discussion casts any doubt on the well established instances where polymorphism in respect of mutual translocations has become a characteristic feature of the genetic architecture of a species, as it has in certain scorpions (Piza—see general review in White, 1954), roaches (John and Lewis, 1959) and, although translocation heterozygotes are much rarer, the mollusc *Thais lapillus* (Staiger, 1955). These are all cases which escape loss of fecundity because they show close to 100 per cent multivalent formation and almost invariable disjunctional orientation at first metaphase.

Selective superiority of translocation heterozygotes must be strongly suspected in such cases. Neither does our hypothesis of "reduplicated chromosome ends" affect in any way the interpretation of occasional individuals heterozygous for translocations (such as the *Trimerotropis gracilis* shown in figure 1 c) which appear from time to time in natural populations as a result of recently arisen chromosomal rearrangements, and which mostly have their fecundity severely reduced, so that the translocation chromosomes are not likely to persist long in the populations.

SUMMARY

Previous workers have interpreted multivalents present at meiosis in the spermatogenesis of hybrid Triturus as due to translocation heterozygosity. An alternative hypothesis according to which these individuals would be homozygous for reduplicated chromosome ends is put forward here.[1]

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[1]After reading the above discussion Professor H. G. Callan writes (*in litt.* May 22, 1961) that he now agrees with the hypothesis of reduplicated telomere regions rather than that of heterozygosity for translocations. He states, in part: "... I have looked at some of Twitty's *Taricha* (Californian) hybrids in male meiosis and once again there are no multivalents in the parents, but reduced chiasma frequencies and multivalents in the hybrids. I was becoming suspicious, and have looked hard at this, and earlier material, for multivalents, such as trivalents, with *non-terminal* chiasmata in *both* arms of the 'common' chromosome. I haven't found a single case. I have looked hard at lampbrush chromosomes of F_1 *cristatus* hybrids, and haven't spotted any obvious translocations—I would have expected to find at least one, since we can recognise many if not indeed most of the chromosome end regions. So I think you are quite right. All newt telomeres are probably homologous... you can quote me on any of the above points."

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

CROSSING OVER WITHIN THE MACROMELANOPHORE GENE IN THE PLATYFISH, *XIPHOPHORUS MACULATUS*•

The platyfish, *Xiphophorus maculatus*, exhibits a high degree of pigmentary polymorphism, consisting in part of a series of patterns composed of large black pigment cells, called macromelanophores. The results from hundreds of crosses made in this and other laboratories have indicated that the appearance and position of these patterns on the body and fins are controlled by a series of dominant multiple alleles. These include *Sd* (spotted-dorsal), *Sr* (striped-side), *Sp* (spotted-side), *N* (black-sided), and *Sb* (spotted-belly). They are located on the sex chromosomes. In this species, some races exhibit a heterogametic male (XX female, XY male) type of sex-determining mechanism, while others exhibit a heterogametic female (WY female, YY male) type (Gordon, 1951).

Two exceptions to this inheritance as classic alleles have been found. In a cross between a WY female and a $Y_{RSp}Y_N$ male (*R*, for red color, is closely linked to *Sp* and *N*) of a "domesticated" strain, Gordon (1937) obtained a *R* female and concluded that *N* and *Sp* were not allelic; however, the possibility that the expression of *Sd* or *N* was surpressed could not be ruled out. Recently, another exception occurred when an XX female was crossed to a $Y_{Sd}Y_{Sr}$ male, resulting in 15 *Sd* males, 28 *Sr* males, and one *SdSr* male. The appearances of the *Sd* and *Sr* patterns were identical in the exceptional fish and its siblings. When it was crossed to an $X_{Sp}X_{Sp}$ female, ten *Sp* females and 15 *SpSdSr* males (triple dominants) were produced. Further crosses confirmed that the *Sd* and *Sr* factors were inherited as a unit.

As Pontecorvo (1958) has pointed out, recent studies have shown that when a sufficiently detailed analysis is made, crossing over within a genic locus is found to occur. In this case, no test of complementarity can be made, since the factors controlling macromelanophore patterns are dominant. Following Pontecorvo's nomenclature, these factors may be described as "sites of mutation," within a macromelanophore gene or as very closely linked genes. Semantics aside, this situation represents a grouping of elements separable by crossing over but closely related functionally.

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A NOTE ON CROSSOVER COINCIDENCE

A paper by S. G. Stephens, titled "A Remote Coincidence," has presented in this issue of the AMERICAN NATURALIST a new look on the classic problem of chiasmata formation and interference (Stephens, 1961). In the course of re-examination of Weinstein's data (1918) on recombination along the entire X-chromosome of *Drosophila melanogaster*, Stephens employed χ^2 contingency tests as the means of detecting the presence of crossover interference. In his approach the magnitude of the χ^2 values was considered as an approximate measurement of crossover interference.

Of course, Dr. Stephens was aware of the classic measure of crossover interference, coincidence value, which takes the value one with no interference and approaches to zero as interference intensifies. The difficulty with this measure of interference was, as he stated, that there was no convenient computing formula for the sampling error of the estimate. There is a classic and very useful book on the measurement of linkage by K. Mather (1951), but we do not find any discussion on this matter. If there has been some work on the subject, many geneticists, including myself, are not aware of its existence.

In an attempt to fill a gap in this problem, I have used the method of maximum likelihood to obtain two crossover values and coincidence value jointly for two segments of a chromosome with their sampling variances. Let two segments of a chromosome be A and B, with p and q for the crossover frequencies, respectively. If c stands for the coincidence value, the expected frequencies of four classes of crossover types, doubles, singles in A and B and non-crossovers, are given as in the second column of table 1. The method leads to the estimators,

$$p = \frac{x + y}{n}, \quad q = \frac{x + z}{n}, \quad \text{and} \quad c = \frac{un}{(x + y)(x + z)}$$

TABLE 1

Experted and observed frequencies of crossover types in two segments of a chromosome

Crossover types	Expected frequencies	Observed numbers
Doubles in A and B	cpq	x
Singles in A	$p(1-cq)$	y
Singles in B	$q(1-cp)$	z
Non-crossover	$1-p-q+cpq$	u
Total	1	n

and their asymptotic sampling variances are,

$$V_p = p(1-p)/n, \quad V_q = q(1-q)/n$$

$$V_c = \frac{c}{pqn}(1 - cp - cq - cpq + 2c^2pq)$$

It is not surprising to note that the sampling variances for p and q are those of binomial distributions. The sampling variance of c is more complex, but

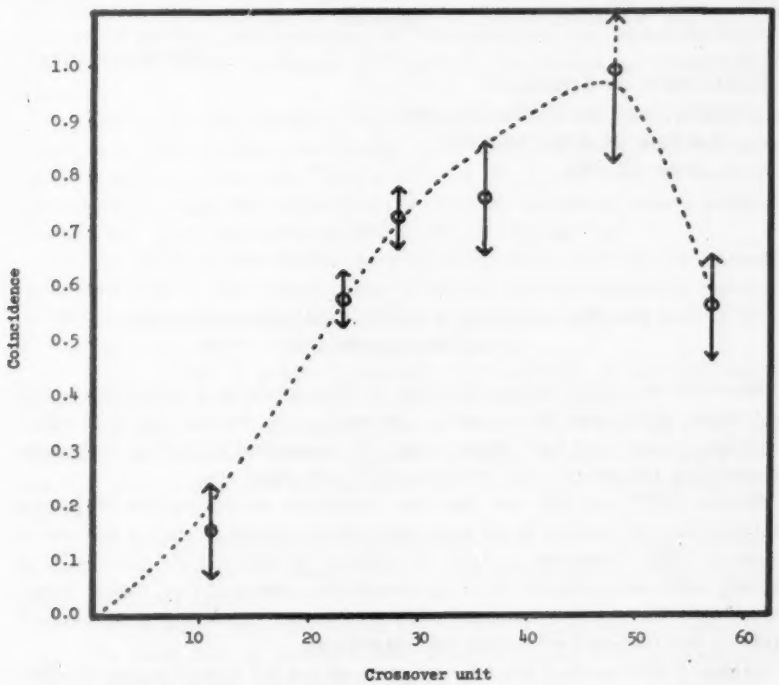

FIGURE 1. The values of crossover coincidence on the X-chromosome of *Drosophila melanogaster* (data taken from Weinstein, 1918). The abscissa represents crossover unit between two segments of chromcsomes involved.

not difficult to compute. For a pair of chromosomal segments with $p = q = \frac{1}{4}$ and $c = \frac{1}{2}$, for example, the expected sampling variance of c would be 6/n, in contrast to 9/n for the same two segments but with $c = 1.0$. Such *a priori* knowledge of the possible range of sampling variances will help to determine the size of experiments on chiasmata formation and interference.

A re-examination of Weinstein's data by the procedure given in this paper leads one to see a clear picture of chiasmata interference as shown in figure 1 which might supplement table 2 in Stephens' article. In this figure the results of analysis are pooled for every five crossover units, and the double-headed arrows represent the standard errors of the respective coincidence values. "A remote coincidence" is plainly observed as the crossover unit increases, and with an extreme distance between two segments, coincidence decreases again.

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A SECOND REGION IN MAIZE FOR GENETIC FINE STRUCTURE STUDIES

Since the discovery of pseudoalleles in Drosophila by Lewis (1951, 1955) and Green (1956) and the recombination studies by Benzer (1957) in bacteriophage, geneticists have been especially interested in finding techniques for studying the genetic fine structure of higher organisms.

Nelson (1957) pointed out that the intralocus recombination of genetic material may be common to all organisms but occurring at such a low rate of frequency that it escapes notice. He further pointed out the feasibility of dealing with the genetics of the gametophytic generation of higher plants rather than the sporophytic generation to increase resolving power comparable to that obtained with some microorganisms.

Nelson (1959) studied the fine structure of the *wx* (waxy) region on chromosome 9 in maize by utilizing the differential iodine staining properties of *wx* and normal pollen. Pollen with *wx* genotype stains red due to the ab-

sence of amylose starch, and *Wx* pollen stains blue due to the presence of amylose.

Nelson's work stimulated our thinking toward the possibility of differentiating other mutants involved in pollen starch synthesis. Studies by Kramer *et al.* (1958) revealed that double recessive *wx ae* (*ae*, amylose extender on chromosome 5) maize kernels contained approximately 15 per cent amylose while the *wx Ae* kernels contained no amylose. A study was undertaken to determine if these characteristics were manifested in the pollen. Later analysis of *wx ae* pollen indicated that it contained approximately ten per cent amylose.

Pollen from a plant homozygous *wx* and segregating *Ae/ae* was studied to determine if there were differences in staining properties. A modification of Nelson's technique (1957) was found necessary. The technique is as follows:

(1) Twenty-four anthers are taken from eight proximal florets on a tassel that has been stored in 70 per cent ethyl alcohol. This usually gives a population size of 50,000 to 100,000 pollen grains.

(2) The anthers are placed in a micro-cup containing one ml. of iodine solution (formula: 13 gms. iodine, 30 gms. potassium iodide, 100 ml. 75 per cent ethyl alcohol, and four drops of Tween 80) and are broken up for three minutes by a VirTis homogenizer, releasing the undamaged stained pollen grains.

(3) The pollen suspension is then filtered through cheesecloth onto filter paper in a suction funnel and washed with deionized water until the excess iodine solution is removed. Then two ml. of 25 per cent ethyl alcohol is run onto the filter paper and allowed to stand for one minute to remove additional iodine stain, after which the alcohol is removed by suction.

(4) The pollen is then washed from the filter paper into a watch glass with deionized water. The watch glass is swirled slightly causing a collection of the pollen grains in the center. The water is then suctioned off by means of a glass tube connected to a vacuum pump.

(5) Next, 0.8 ml. of gelatin medium (0.5 gm. gelatin, 25 ml. H_2O, and two drops Tween 80) is added to the pollen in the watch glass.

(6) The medium is then poured onto a large glass slide (80 × 100 mm.). The pollen is uniformly distributed over the slide. A large coverslip (50 × 75 mm.) is placed over the pollen with care to prevent the trapping of air bubbles.

(7) The freshly prepared slide is then heated at 50°C for eight minutes in an oven. This treatment changes *wx Ae* pollen from black to red, the *wx ae* pollen remaining black.

(8) After heating, the coverslip is sealed with a slide ringing compound.

(9) The slide can be scored at this time or placed in a refrigerator and scored later for differential staining types.

Since we do obtain differential staining characteristics between *wx ae* and *wx Ae* pollen, this may allow a study of recombination at the *ae* region simi-

lar to Nelson's study of the *wx* region when different sources of *ae* are crossed in a *wx* background and the F_1 pollen is observed.

It has been observed that the pollen in some *wx ae* lines stains very black while other lines stain much lighter. It would seem necessary to select parental lines that stain very dark for best results in recombination studies. The reds are difficult to differentiate in a light staining background. A range of 0.04 to 0.1 per cent red-staining pollen grains has been observed in the standard *wx ae* line. These values were derived by making complete counts of the red-staining grains and estimating the total population by counts made at eight randomly selected areas. The knowledge of natural occurring reds is necessary as it indicates the background which one must consider in detecting low recombination frequencies.

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